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JOURNAL OF ANATOMY

AN EARLY HUMAN EMBRYO, WITH 0·55 MM. LONG EMBRYONIC SHIELD

By T. B. JOHNSTON

Guy's Hospital Medical School

Despite the fact that our knowledge of the normal development of the human embryo in its early stages has increased very substantially in the past twenty years, there are still many points on which further evidence is necessary and which cannot be elucidated until a much larger amount of material is available. The embryo on which this communication is based is a well-fixed specimen, and on this account a description of its main features may possibly contribute a little towards the solution of some of the outstanding problems.

The embryo represents a slightly later stage of development than the beautifully preserved Strahl-Beneke (1910) specimen, and a slightly earlier stage than the Embryo Hugo (1926), with both of which it will be compared specifically. It corresponds in many ways to Grosser's (1931*b*) Embryo H.Schm. 10, but as, at the time of writing, no detailed description of this embryo has been published and only the figure of a schematic median section is available, I can use it only occasionally for purposes of comparison. The subject of this communication will be referred to as H.R. 1, as it was obtained at operation by Mr E. Hesketh Roberts, to whom I am very greatly indebted for the specimen and for the following clinical notes on the case.

HISTORY

The specimen was obtained from the uterus of a woman on whom hysterectomy was performed. She attended the Gynaecological Clinic of St John's Hospital, Lewisham, in February 1932, complaining of pain in the right iliac and sacral regions. She was found to be suffering from congestive dysmenorrhoea, with mild menorrhagia and marked leucorrhoea, which dated from her last confinement, two years previously, when she had given birth to healthy twins. Prior to that confinement her periods had always been regular every 28 days and had lasted for 5 days, but since then they had increased in duration to 6 or 7 days, although the intermenstrual period remained unaltered. There was no history of previous miscarriages. On examination the uterus was found to be moderately enlarged and the condition was diagnosed as either multiple small fibromyomata or chronic subinvolution, accompanied by an early stage of cystocele. It was decided to perform a subtotal hysterectomy, combined

with abdominal cystopexy and she was admitted to hospital on 12 July 1932. The first day of the last menstrual period was 17 June. The operation was performed on 14 July. A corpus luteum was found in the right ovary, and, when the uterus was opened after removal, the endometrium was found to be generally and uniformly thickened and congested. It contained a haemorrhagic-looking spot about the size of an ordinary pin's head. The muscular wall of the uterus was slightly thickened. The whole organ was fixed in 5 % formalin and subsequently the haemorrhagic spot was removed for section together with the whole thickness of the underlying uterine wall. A few sections were cut and showed the presence of an early chorionic vesicle. Unfortunately, the block was mislaid for some time and it did not come into my possession until November 1935, when it was no longer possible to obtain any further history or the dates of coitus.

TECHNIQUE

It proved necessary to reblock the specimen. This was especially unfortunate as the chorionic cavity had already been opened, but the condition of the block left no option. The fresh block was cut into a perfect series of sections at 5 μ, and the sections were stained with Mayer's haemalum and alcoholic eosin.

The sections were then drawn at a magnification of 200 and a reconstruction model was made in millimetre board.

The plane of section was rather unfortunate. At first I estimated that it made an angle of 10° with the cranio-caudal axis, but I am now of opinion that the angle is not much more than 5° so that the sections are nearly longitudinal (Text-fig. 1 *a*). In addition, however, the section plane made an angle of approximately 57° with the horizontal plane (Text-fig. 1 *b*), and this obliquity has increased the difficulties of interpretation, especially at the caudal end of the shield.

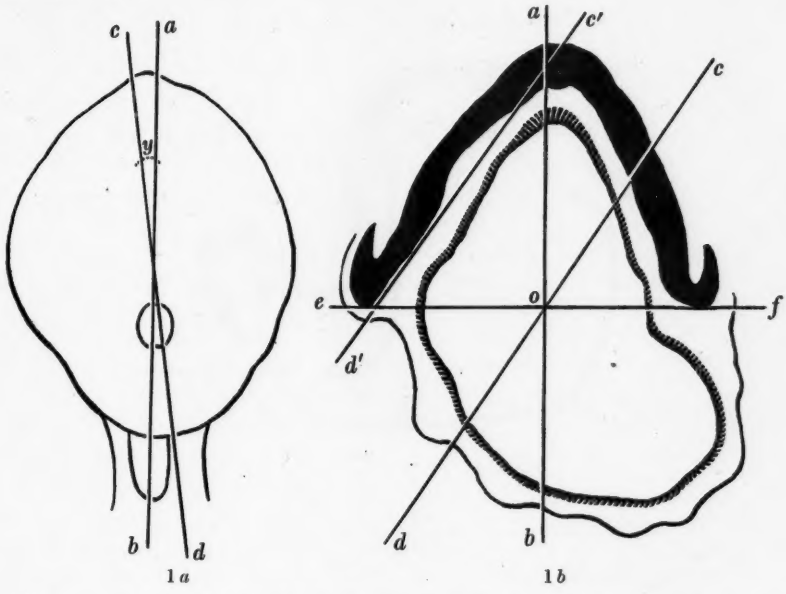
THE OVUM *IN SITU*

Before the individual features of the embryo are described, a brief reference may be made to the appearance of a section through the whole ovum *in situ*. Such a section is shown in Pl. 1, fig. 1.

The ovum is not deeply implanted but, like the Strahl-Beneke (1910), the Embryo Hugo (Stieve, 1926) and many others, projects beyond the surface of the uterine mucosa. The amount of projection measures nearly 2 mm. beyond the level of the surrounding endometrium. An unusually large blood clot covers the central part of the decidua capsularis and obscures the original point of entry. The chorionic cavity is almost triangular in the section, with the slightly blunted apex farthest away from the surface of the mucosa. As usual, the embryo lies in the deepest part of the cavity and its connecting stalk is attached in the apex of the triangle, although this connexion is not shown in the figure.

The implantation is restricted to the stratum compactum, which shows numerous patches of localized oedema. One of the large venous sinuses, described by Bryce (1908), Falkiner (1932), Teacher (1924) and others, is seen deep to the decidua basalis, in the interval between the stratum compactum and the stratum spongiosum. It is cut obliquely. The stratum spongiosum shows the structure characteristic of an early pregnancy. The glands are enormously dilated and full of secretion, and their epithelium shows the familiar "saw-teeth" projections. A few of the glands in the neighbourhood of the ovum contain blood.

A fuller account of the endometrium will be given in a subsequent communication.



Text-fig. 1 *a*. Schema of the dorsal projection of H.R. 1. The line *ab* is the median plane and *cd* is the section plane. The angle $y = 5°$.

Text-fig. 1 *b*. Schema of a transverse section through H.R. 1. The line *ab* is the median plane and *cd* is the section plane. The angle $cof = 57{\cdot}5°$. The line *c'd'* represents approximately section 20·10.

RECONSTRUCTION MODEL

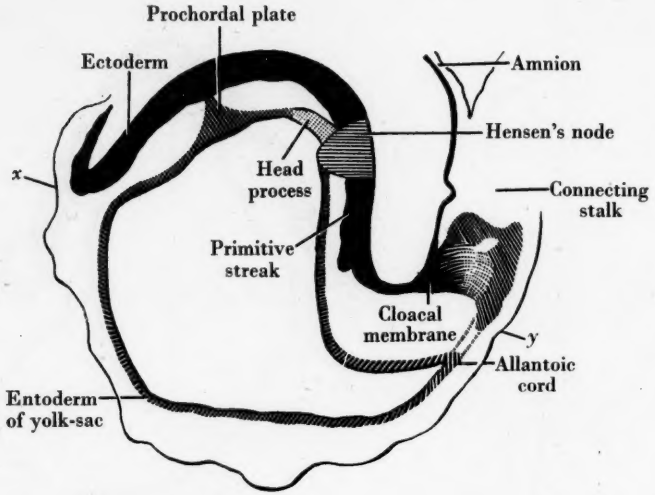
As stated already, a reconstruction model of the embryo was made in millimetre board at a magnification of 200. It included the connecting stalk and a portion of the chorion but not the roof of the amnion, which had collapsed on the shield in places.

The most striking feature of the outside of the model is the shape of the

embryonic shield. It is, as usual, more or less oval in outline, but is convex dorsally in all diameters. A slight degree of dorsal convexity is not uncommon in the early human embryo, but the degree of curvature is much greater in H.R. 1 than it is in the Bi. 1 (Florian, 1927), the T.F. (Florian, 1928), and the Thompson-Brash (1923) embryos. It is a matter of considerable importance and will receive full consideration at a subsequent stage.

A second, but less important, feature is the presence of a thickened rim of amniotic ectoderm round the cranial and lateral borders of the shield.

The median section of the model shows that the roof of the yolk-sac bulges up into the concavity of the ventral surface of the shield (Text-fig. 2). At



Text-fig. 2. Drawing of the median section through the reconstruction model of H.R. 1, partially idealized. The roof of the amnion has been removed. × 150. The line *xy* represents the plane of the dorsal projection shown in Text-fig. 1 *a*.

first sight its cavity does not appear to be disproportionately small compared with the size of the embryo. This appearance is misleading and, if the shield were flat, or approximately flat, the small size of the yolk-sac, as witnessed by its measurements (Table 1), would be a very obvious feature.

A wide funnel-shaped diverticulum projects from the caudal surface of the sac and gives off from its summit a relatively long entodermal cord which passes into the connecting stalk (Text-fig. 2). This solid cord of cells is identified as the primordium of the allantoic canal.

Hensen's node, which is seen in the median section of the model, lies caudal to the middle of the shield. Its position corresponds fairly closely to the position of the anterior end of the primitive streak in the Strahl-Beneke

Table 1. *Principal measurements of the human embryos to which special reference is made in the text*

Embryo	Cavity of chorion	Shield L.	Shield W.	Hensen's node	Primitive streak	Head process	Cloacal membrane	Allantois	Prochordal plate	Cavity of yolk-sac	Villi Caps.	Villi Marg.	Villi Bas.
Fetzer	1·56 × 1·048	0·26	0·215	.	0·05	.	+	.	.	0·18 × 0·15 × 0·15	0·18	0·31	0·14
Strahl-Beneke*	2·2 × 2·2 × 1·2	0·37	0·23	.	0·1	.	+	0·093	0·066	0·46 × 0·34	.	.	.
Meyer	2·6 × 2·1 × 2·7	0·41	0·40	.	0·12	0·06?	?	.	.	.	0·05	.	0·2
T.F.	3·07 × 4·5 × 1·7	0·4	0·43	.	0·162	.	+	.	.	0·24 × 0·25 × 0·25	0·18	0·6	0·45
H.Schm. 10	.	0·51	0·58	0·1	0·14	0·1	+	0·08†	.	0·9 × 0·7 × 0·45	.	.	.
H.R. 1‡	1·95 × 1·3 × 1·8	0·55 (0·48)	0·43	0·06	0·055 (0·06)	0·04	0·04 (+)	0·135	0·075	0·35 × 0·23 × 0·3	0·2	0·3–0·6	0·6
Hugo	4·4 × 4·7 × 3·8	0·57	0·63	0·06	0·24	0·09	0·035§	?	–	0·52 × 0·64 × 0·53	0·3	.	1
Bi. 24	3·05 × 3·036 × 3·029	0·62	0·41	0·05	0·28	0·1	0·06	0·15	0·03	.	.	.	.
Manchester	.	0·87	0·62	0·05	0·44	0·12	0·06	0·21	0·03	0·98 × 0·74 × 0·98	.	.	.

The measurements given are those published by the authors concerned except in the cases noted.

* All measurements as given by Florian, J. (*Anat. Anz.* Ergänz-zum, **71**).

† Measured by T. B. J. from the author's figure.

‡ The measurements given in brackets were made by T. B. J. on Florian's figure (Text-fig. 35).

§ Measured by Florian, J. (*Philos. Trans.* B, **219**, 480).

embryo, although, prior to the formation of the head- and tail-folds, it is usually situated at or about the middle of the shield. The part of the primitive streak caudal to the node is short and exhibits an early stage of differentiation. It does not extend to the caudal limit of the shield. The head process is seen extending cranially for a short distance from the deep part of the node.

Cranial to the head process the roof of the yolk-sac shows a patch of thickened entoderm (Text-fig. 2), and evidence will be brought forward to support the view that it represents the primordium of the prochordal plate.

One other feature, visible on the median section of the model, should be mentioned at this stage. The entoderm of the distal end of the allantoic cord comes into continuity with the ectoderm of the amnion immediately caudal to the shield (Text-fig. 2). This represents the cloacal membrane (p. 38).

The connecting stalk passes from the caudal end of the embryo to the chorion. On its right side, close to its embryonic attachment, it is marked by a deep groove, which is continuous at its right extremity with a tear into the amnion. This groove, owing to the nature of its walls, was regarded at first as a natural feature, but further consideration, prompted by a suggestion from Prof. Florian, led to the conclusion that it was an accidental tear. This discovery served to remove two difficulties which have not yet been mentioned. (1) The attachment of the allantoic cord to the yolk-sac lies considerably to the left of the median plane, as will be shown in the description of the individual sections, and the cord itself shows an angled bend. (2) The yolk-sac is not symmetrically disposed with reference to the median plane, but projects more to the left side than to the right.

The identification of the groove as an accidental tear made it clear that the embryonic end of the connecting stalk had become slightly kinked on the rest of the stalk and had swung the more ventral part of the yolk-sac over to the left.

BRIEF DESCRIPTION OF THE INDIVIDUAL SECTIONS

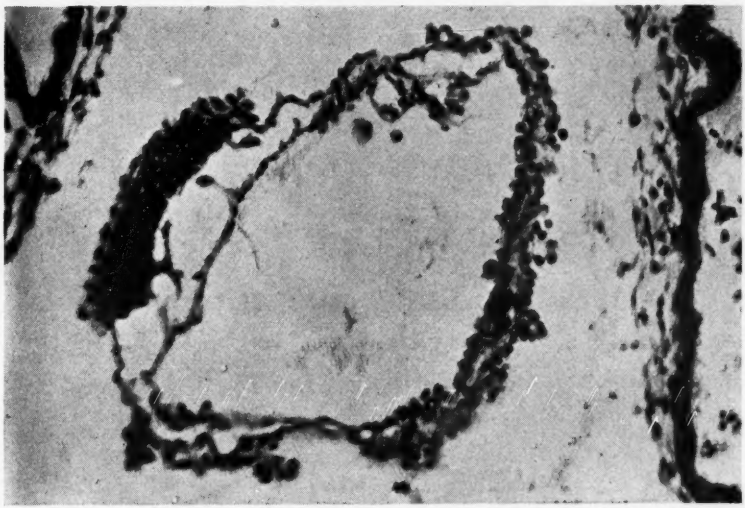
The sections pass through the embryo from its left to its right side. Owing to the obliquity of the section plane (p. 2), the sections pass at first through the left and ventral walls of the yolk-sac, and it is not until the twenty-first section is reached that the embryonic shield appears. Owing to the angulation with the median plane (Text-fig. 1) the sections cut the median plane of the shield at its caudal end first. The individual slides are numbered serially and twelve sections are mounted on each.

The first section through the primary mesoderm on the left wall of the yolk-sac appears in 16·4, but it is not until 16·10 that the cavity of the sac is opened into freely. The left edge of the amnion appears in 17·12, and the edge of the embryonic shield in 18·1. Sections 16·4–17·12 are not figured.

18·1 (Text-fig. 3). The left edge of the embryonic shield and the left margin of the amnion are seen but the amniotic cavity is not yet opened. It lies in the figure to the left of the yolk-sac, which shows a short, wide diverticulum from

the ventral end of its caudal wall (upper end in the photograph). Blood islands are present on the ventral and cranial walls of the sac. The entodermal cells show a variety of forms.

18·3 (Text-fig. 4). The amniotic cavity is apparent and the caudal diverticulum of the yolk-sac has increased in length. A few strands of intra-embryonic mesoderm are apparent. In view of the condition found in later sections it should be noted that the primary mesoderm on the cranial end (lower end in the photograph) of the amnion passes straight on to the surface of the yolk-sac.

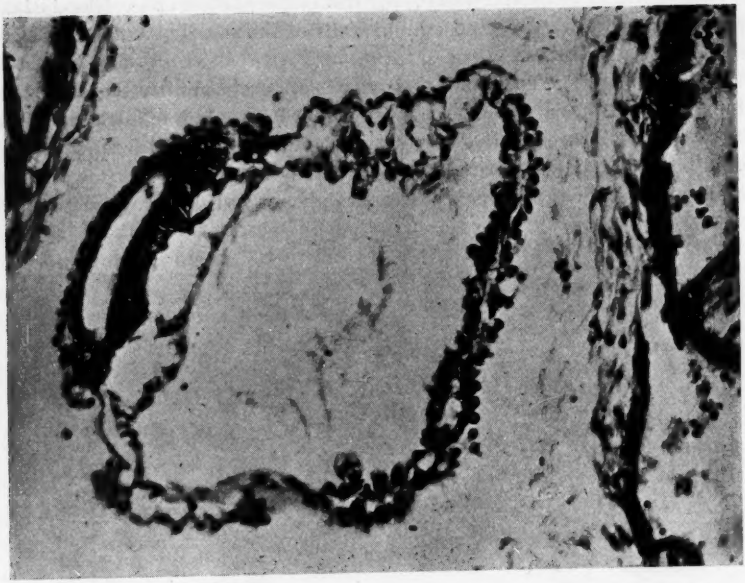


Text-fig. 3. Section 18·1. ×200.

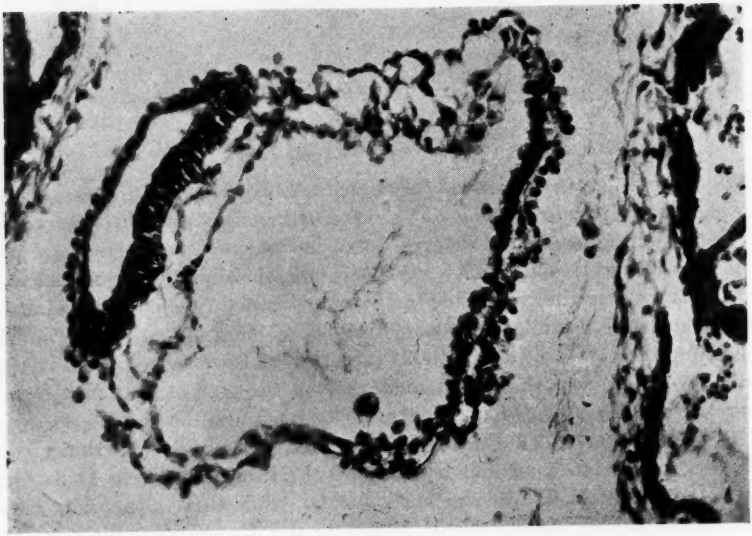
18·4 (Text-fig. 5). The shield has elongated, especially at its caudal end. The caudal diverticulum of the yolk-sac is a little longer and a little narrower.

18·5 (Text-fig. 6). Both changes noted in the preceding section are progressing. In addition the intra-embryonic mesoderm forms an almost continuous layer over the caudal half or more of the embryonic area, and at its caudal limit its cells are continuous with those of the primary mesoderm. The cells lining the yolk-sac are of at least three different types: (*a*) on the ventral wall they are cubical or low columnar; (*b*) on the cranial wall they form a syncytial ribbon; (*c*) in the roof they are elongated and flattened.

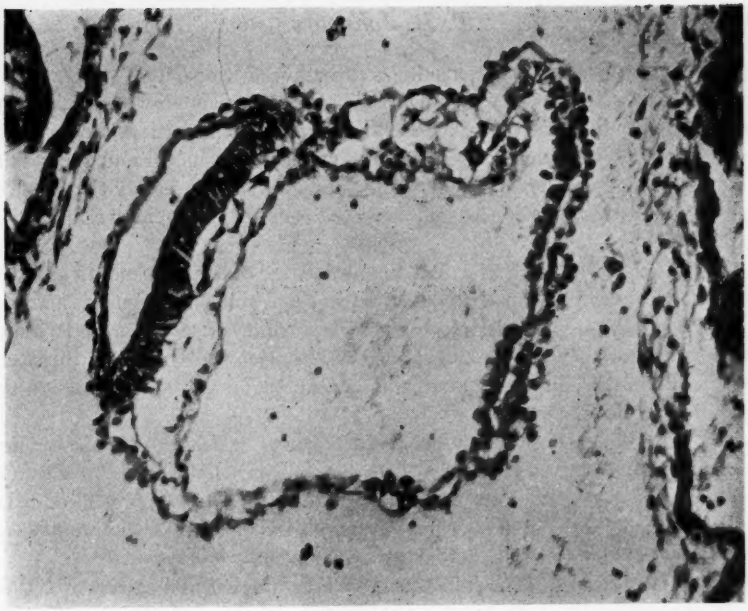
18·6 (Text-fig. 7). The caudal diverticulum of the yolk-sac is longer and narrower and shows a distinct bend, concave dorsally. The cells at its apex and on its ventral wall are large and cuboidal, while those on its dorsal wall are more elongated and flattened. Dorsal to the diverticulum the entoderm on the caudal wall of the yolk-sac shows an area of apparent thickening. This



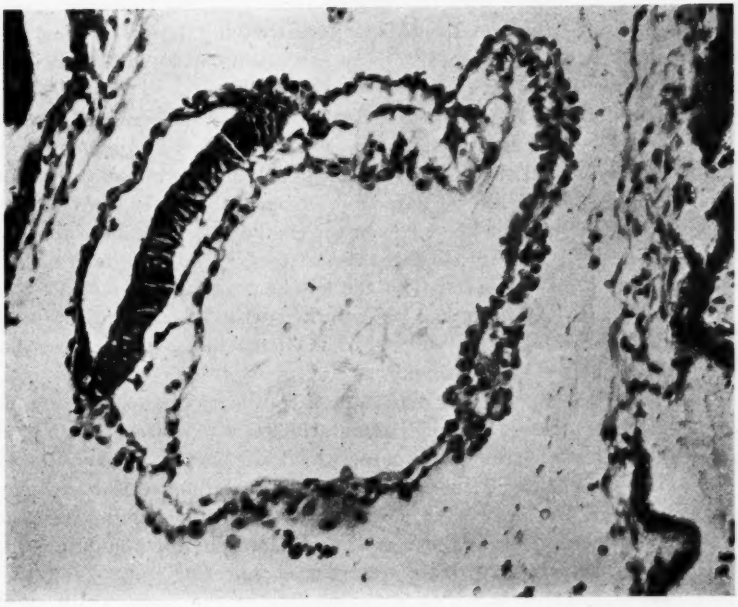
Text-fig. 4. Section 18·3. ×200.



Text-fig. 5. Section 18·4. ×200.



Text-fig. 6. Section 18·5. ×200.



Text-fig. 7. Section 18·6. ×200.

appearance is repeated in the ensuing four or five sections, and is regarded as being due to a wrinkling of the wall of the yolk-sac associated with the kinking due to the tear in the connecting stalk. A similar explanation is offered for the additional cells which lie between the primary mesoderm and the caudal wall of the yolk-sac. The intra-embryonic mesoderm covers nearly the whole of the embryonic area, and consists of elongated cells with branching and anastomosing processes.

18·8 (Text-fig. 8). All the features noted in 18·6 are present, and the indications of wrinkling in the caudal wall of the yolk-sac are more marked. The intra-embryonic mesoderm establishes continuity with the primary mesoderm at the cranial end of the shield. In this situation the exocoelom threatens to encroach on the embryonic area, and the encroachment becomes real in the succeeding sections.

18·9 (Text-fig. 9). At its caudal end the shield ectoderm and the amnion are cut obliquely, foreshadowing a further extension of the shield in a caudal direction. The part of the caudal diverticulum of the yolk-sac which lies distal to the bend already noted has become much narrower. At the cranial end of the shield the exocoelom encroaches into the embryonic area.

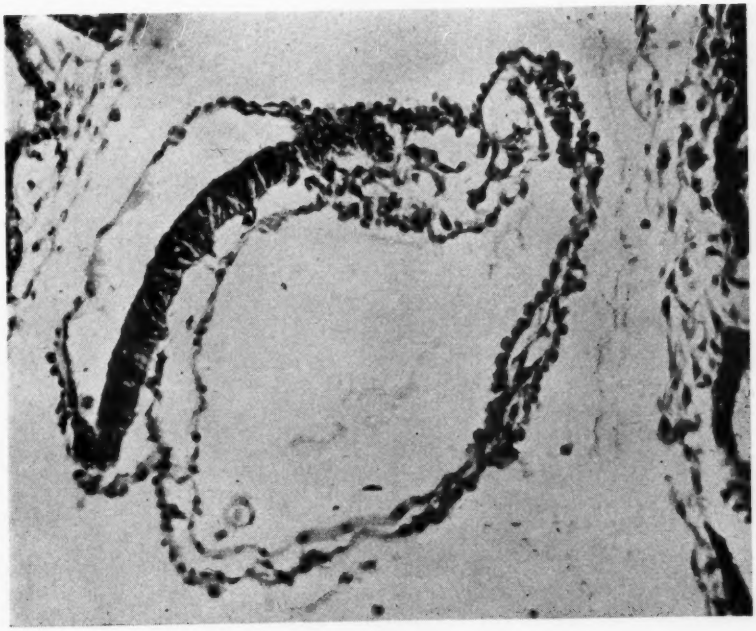
18·10 (Text-fig. 10). The shield has extended farther in a caudal direction, and is cut obliquely at its caudal end; in this situation the shield ectoderm appears to be contributing to the formation of the intra-embryonic mesoderm. The narrow recess at the distal end of the caudal diverticulum of the yolk-sac has just lost its connexion with the interior of the sac. In the cranial part of the sac a group of entodermal cells is apparently lying free; they are part of a large wrinkle in the cranial wall of the sac which can be identified in the succeeding six sections.

18·11 (Text-fig. 11). A further extension of the shield in the caudal direction is apparent. A large patch of cells, clearly ectodermal in origin, lies ventral and caudal to the caudal end of the shield. In part this patch represents an oblique shaving through the shield ectoderm, but in part it appears to be forming intra-embryonic mesoderm. The recess of the caudal diverticulum of the yolk-sac has disappeared; its apex has given rise to a solid cord of entodermal cells which is cut transversely as it lies in the left part of the attachment of the connecting stalk to the embryo. Well-marked wrinkles are present in the cranial and caudal walls of the yolk-sac. The encroachment of the exocoelom on the embryonic area is quite definite.

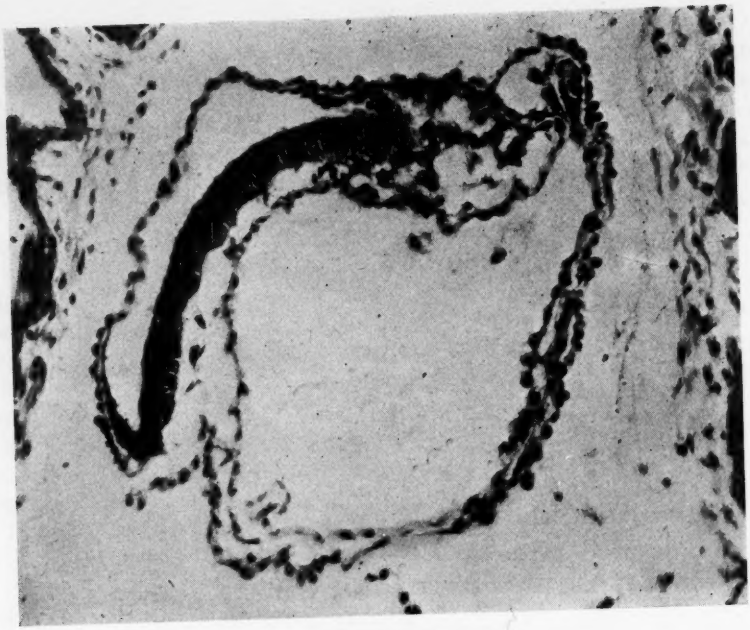
18·12 (Text-fig. 12). Further extension of the shield in a caudal direction is indicated by the oblique shaving through the left wall of the amnion at the caudal end. The solid entodermal cord, which is regarded as the allantoic representative, is seen cut transversely, as in the preceding section. Above it in the figure a few cells are visible; they are part of the connecting stalk, and the interval between them and the part which contains the allantoic cord is due to the bend associated with the tear in the connecting stalk.

19·1 (Text-fig. 13). Owing to an inequality of the floating out of the

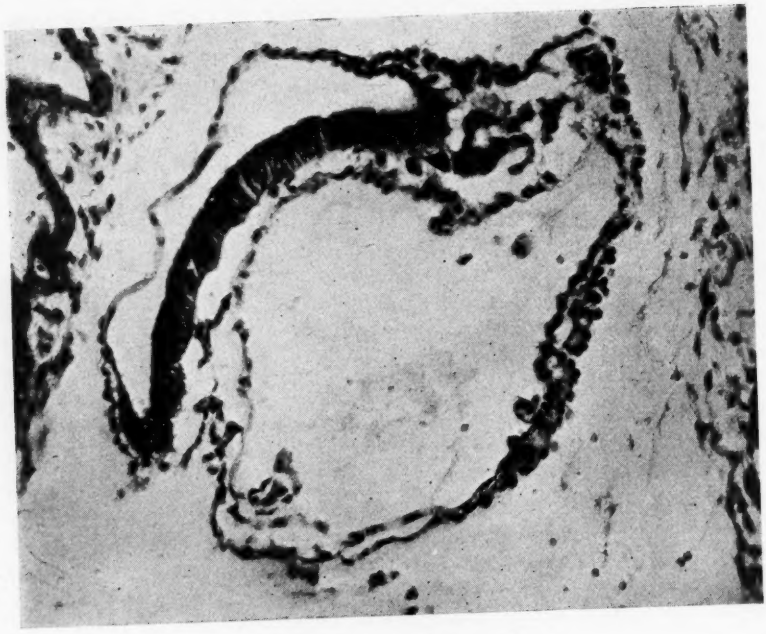
Text-fig. 8. Section 18·8. ×200.



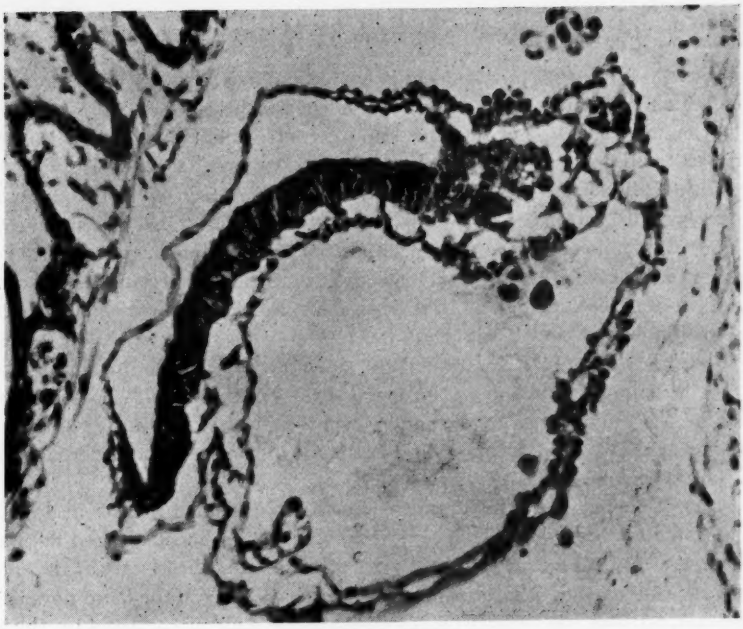
Text-fig. 9. Section 18·9. ×200.



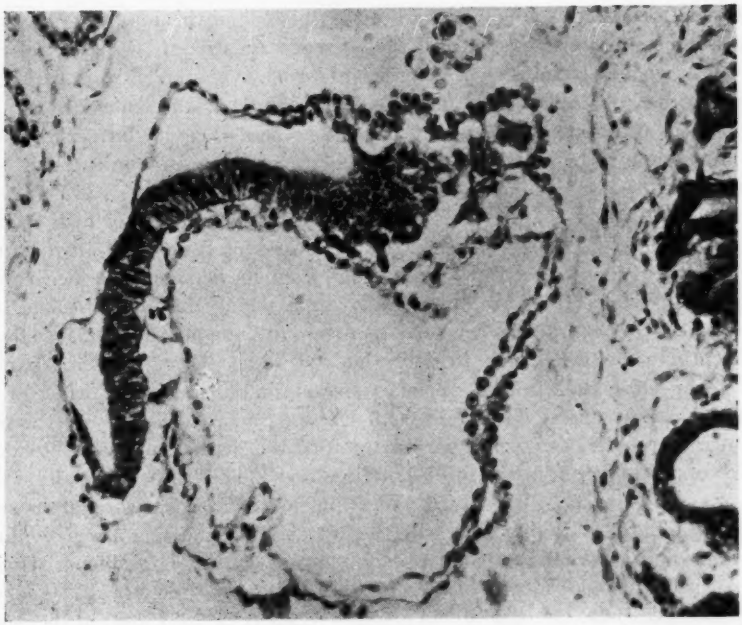
Text-fig. 10. Section 18·10. ×200.



Text-fig. 11. Section 18·11. ×200.



Text-fig. 12. Section 18·12. ×200.



Text-fig. 13. Section 19·1. ×200.

sections the curvature of the shield is less pronounced on slide 18 than it is on slide 19 and the succeeding slides. The shield continues to extend in a caudal direction, and the edge of the shield and the left wall of the amnion are again cut very obliquely at the caudal end of the embryo. In this situation two groups of cells are seen growing out from the deep surface of the ectoderm and occupying the gap between it and the roof of the yolk-sac. Their significance is uncertain. The condition of the connecting stalk is unchanged. It still consists of two portions, one of which (the lower in the figure) contains the allantoic cord. At the cranial end of the embryonic area the encroachment of the exocoelom is deep and >-shaped.

19·2 (Text-fig. 14). No important changes are present in this section. A small, shallow depression on the dorsal surface of the shield, which is present also in the succeeding section and then disappears, has no ascertainable significance. Two mesodermal cells are present in the >-shaped encroachment of the exocoelom.

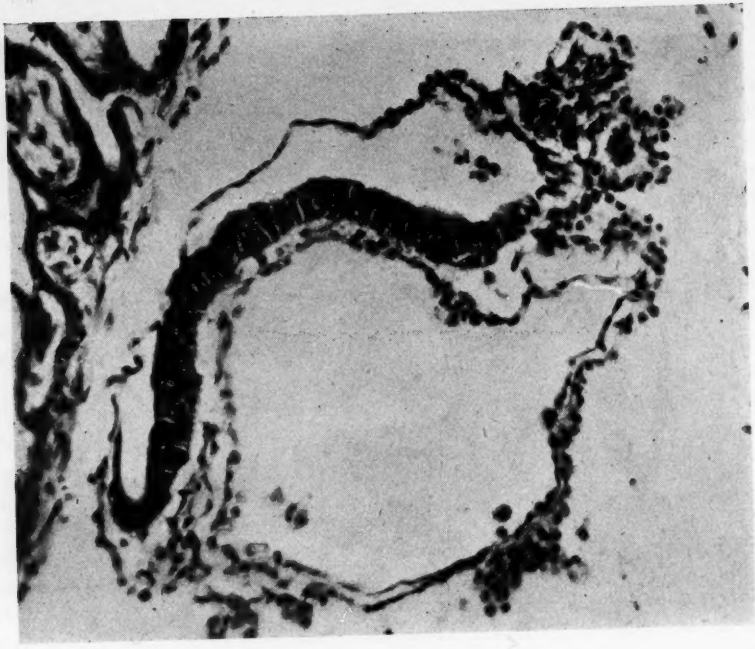
19·3 (Text-fig. 15). The amniotic cavity has extended further in a caudal direction and the caudal extension of the shield has almost reached its maximum. A beautiful mitotic figure is present in the superficial part of the shield near its caudal end. The two parts of the connecting stalk have now come together, so that the section passes through the floor of the bend on the left surface of the stalk. In this section the roof of the amniotic cavity in its middle third shows an appearance almost identical with the appearance of the ventral part of the cranial wall of the yolk-sac in this and other sections. It is impossible to satisfy oneself that it really consists of two layers of cells for the two appear to be fused together to form a syncytial ribbon. Reference will be made to this appearance at a later stage. At the cranial end of the shield the intra-embryonic mesoderm shows traces of cavity formation and it is impossible to be certain whether or not the spaces communicate with the exocoelom.

19·4 (Text-fig. 16). Near its caudal limit the ventral surface of the shield ectoderm loses its sharp contour, which is so striking in its cranial two-thirds, and a small mass of cells is growing out from its deep surface in a caudal direction. The sections are now rapidly approaching the median plane, and these cells are regarded as derivatives of the end-node of the primitive streak. Attention should be drawn to the irregularly <-shaped gap which separates the connecting stalk from the primary mesoderm on the dorsal surface of the caudal diverticulum of the yolk-sac. A few detached cells occupy the gap, which represents the deepest part of the furrow described on the right side of the connecting stalk in the reconstruction model. The gap is more obvious in the later sections and has already been interpreted as an accidental artefact. At the cranial end of the shield the intra-embryonic mesoderm contains further and more obvious signs of cavity formation. A large blood island is present on the ventral wall of the yolk-sac.

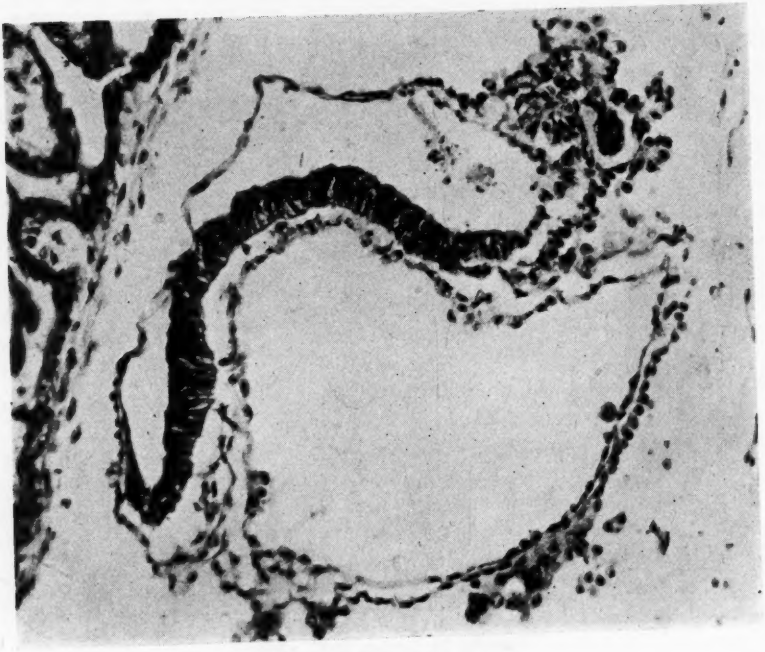
19·5 (Text-fig. 17). The shield has now reached its full extent at its caudal

Text-fig. 14. Section 19·2. ×200.

Text-fig. 15. Section 19·3. ×200.



Text-fig. 16. Section 19·4. ×200.



Text-fig. 17. Section 19·5. ×200.

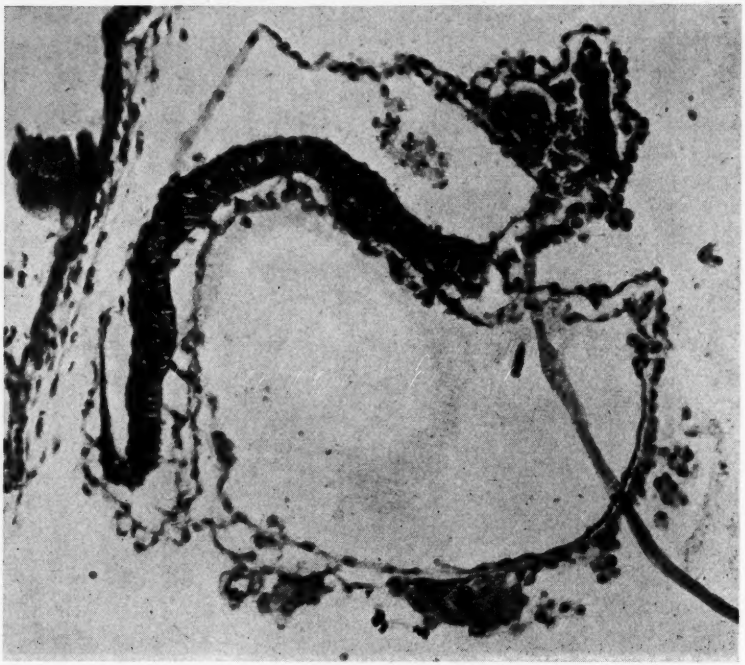
end, and it is to be noted that in that situation the shield ectoderm consists of only a single layer of cuboidal cells. The end node of the primitive streak is seen 0·08 mm. from the caudal end of the shield. Its constituent ectoderm is thicker than the ectoderm of the rest of the shield and its ventral border is irregular, showing no trace of a basement membrane. There is no break in the continuity of the underlying entoderm. From the caudal end of the node cells are growing caudally into the connecting stalk, the tear in which is now more obvious. The allantoic cord is now cut obliquely as it bends towards the

Text-fig. 18. Section 19·6. ×200.

section plane. At the cranial end of the shield the intra-embryonic mesoderm shows extensive cavity formation and its continuity with the primary mesoderm is again obvious. The exocoelom no longer encroaches on the embryonic area. A small vascular space, best seen in 19·6, is present in the blood island on the ventral wall of the yolk-sac.

19·6 (Text-fig. 18). At its caudal end the shield is now reduced to a single layer of flattened cells, over an area 0·05 mm. long. The end node of the primitive streak and the cells which it contributes to the connecting stalk are clearly seen. The allantoic cord is now cut in its long axis, or nearly so. As before, its outline is sharp except at its ventral end where there is an appear-

ance which suggests active growth towards the ectoderm in the angle between the amnion and the extreme caudal limit of the shield. This is the first indication of the cloacal membrane. At the cranial end a large cavity is now apparent in the intra-embryonic mesoderm. Cranially its walls meet and become continuous with the primary mesoderm. This cavity is regarded as a prococious coelomic formation. Caudal to it a small patch of thickened entoderm is adherent to the basement membrane of the shield ectoderm.



Text-fig. 19. Section 19·7. × 200.

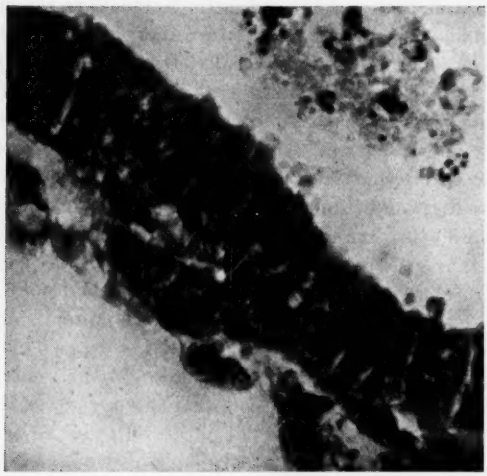
19·6 (Pl. 1, fig. 2). The precocious coelomic cavity and the adjoining parts of the embryo are shown at a magnification of 950. It is significant that whereas traces of cytodesmata are visible connecting the one wall to the shield ectoderm and the other to the roof of the yolk-sac, there is no evidence of any such connexions across the cavity itself. With the exception of one rounded cell all the cells in the walls of the cavity are elongated and typical mesoblasts.

19·7 (Text-fig. 19). The flattened cells at the caudal end of the shield are shrunken and in the underlying mesoderm a small gap indicates a continuation of the tear which in the ensuing sections breaks through into the amniotic

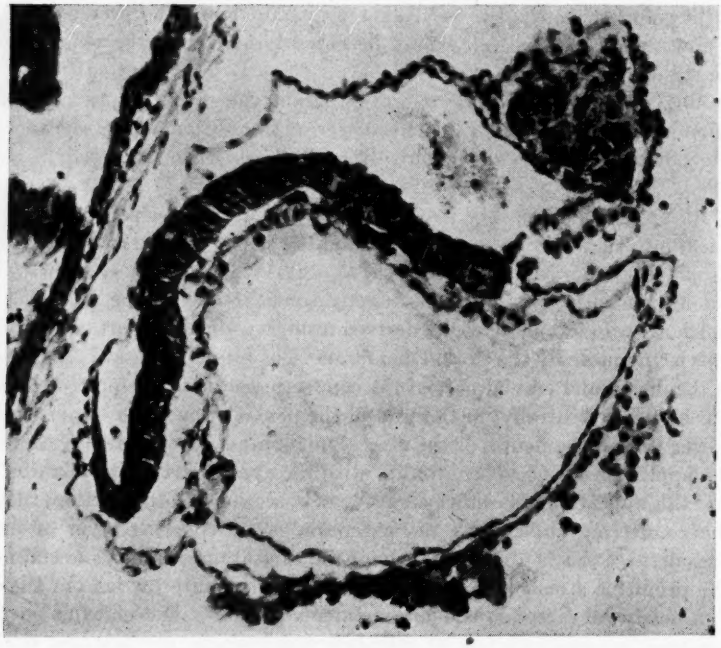
cavity. The allantoic cord is again cut in its long axis in the connecting stalk. Its apex and caudal (right-hand) border are sharply delimited from the overlying primary mesoderm, but its cranial (left-hand) border, especially in its ventral (lower) part, is very indefinite, and in this region the entodermal cells are in continuity with the cells which are interposed between them and the amniotic cavity and which are regarded as a thickening of the amniotic ectoderm. This ecto-entodermal continuity is identified as the cloacal membrane. In the dorsal and cranial (upper and left-hand) part of the connecting stalk a rounded mass of cells, suggestive of a large blood island, also represents part of the ectodermal constituent of the cloacal membrane, as will be apparent in the succeeding two sections. Cells derived from the end node of the primitive streak are still apparent and, slightly nearer to the cranial end, a large mass of cells is growing headwards from the primitive streak. This mass, which is 0·03 mm. long, seems to be devoid of entoderm on its ventral surface. It is equally conspicuous in the next section, scarcely recognizable in the next but one, and thereafter disappears. Cranial to this outgrowth the shield is slightly thickened and its ventral surface is irregular and woolly. This represents the first shaving through the left border of Hensen's node. At the cranial end of the shield the precocious coelomic cavity is again conspicuous, and caudal to it the entoderm of the yolk-sac is adherent to the basement membrane of the shield ectoderm, although no thickening is apparent. The presence of a desquamated entodermal cell should be noted lying free in the yolk-sac below the middle of the shield.

19·7 (Text-fig. 20). The outgrowth from the primitive streak and the adjoining shaving through the left border of Hensen's node are shown × 684. The outgrowth is obviously directly continuous with the ectoderm of the streak.

19·8 (Text-fig. 21). The tear at the caudal end has now passed through the shield into the amniotic cavity. The allantoic cord is not so easy to recognize. Its caudal border (right border in the figure) is still sharp except at its middle, but its ventral border becomes directly continuous with the adjoining cells, which are ectodermal in origin, derived from the adjoining part of the amnion. This is undoubtedly the cloacal membrane. The rounded mass of cells observed in the dorsal and cranial part of the connecting stalk in the preceding section is continuous ventrally, in this and in the next section, with the ventral part (lower part in the figure) of the cloacal membrane. At its narrow, caudal end the appearance suggests continuity with the apical part of the allantoic cord, and this appearance is confirmed in the next section. In this section, therefore, the connecting stalk, with the exception of its covering layer of primary mesoderm, consists almost entirely of the cloacal membrane. The end node of the primitive streak is no longer visible, but the outgrowth from the streak in a headward direction is again apparent. At its cranial end this outgrowth reaches the caudal surface of Hensen's node, which now involves the whole thickness of the embryonic area. The sharp ventral contour of the shield

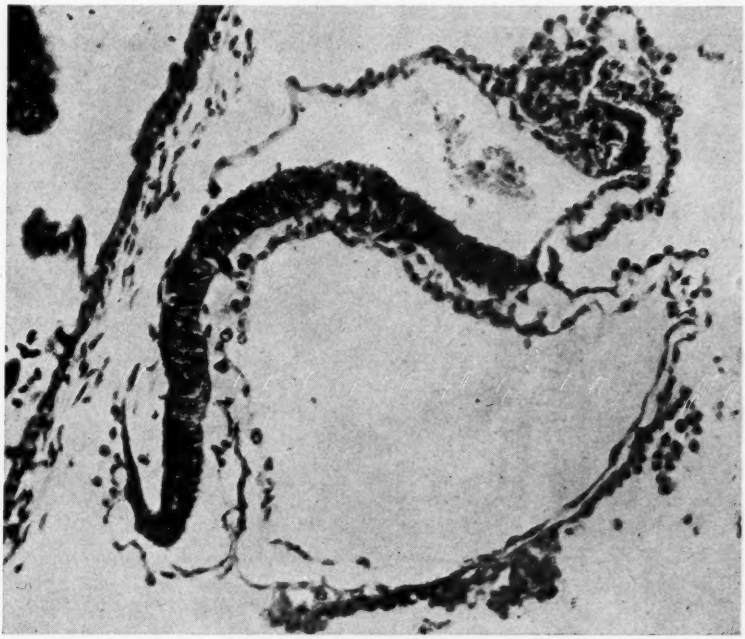


Text-fig. 20. A portion of section 19·7, showing the region of the primitive streak. ×634.



Text-fig. 21. Section 19·8. ×200.

ectoderm stops very abruptly at the cranial limit of the node. The characteristic structure of the node is seen better in sections 19·10 and 19·11. The condition of the roof of the yolk-sac immediately cranial to the node is difficult to interpret. It is formed by a number of low columnar cells which appear to be continuous caudally with the deep part of the node. Attention should be drawn to a large, round entodermal cell, with a deeply pycnotic nucleus, which is lying free in the yolk-sac in this situation, and to the fact that more cranially the entoderm seems to stop abruptly at a cell which shows

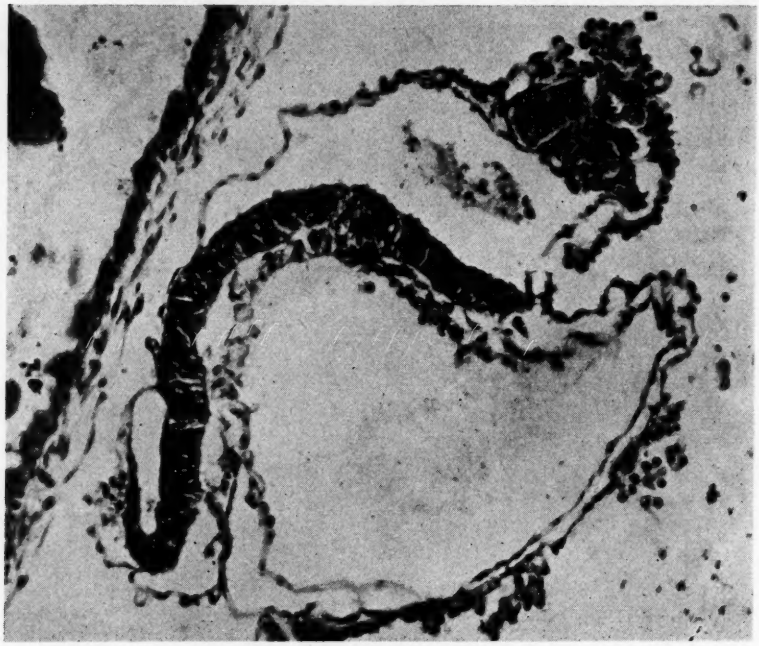


Text-fig. 22. Section 19·9. ×200.

a good mitotic figure. The low columnar cells are regarded as forming the left edge of the primordium of the head process. Between them and the ectoderm a group of five mesodermal cells is interposed. These cells really lie to the left of the head process but appear to lie dorsal to it owing to the obliquity of the section plane. At the cranial end of the shield the coelomic cavity, though present, is in two parts and is not so convincing as it is in the two preceding sections. Caudal to it the entoderm is still adherent to the shield ectoderm, although there is no localized thickening.

19·9 (Text-fig. 22). In the connecting stalk the entoderm of the allantoic cord is now cut in two places. The larger, proximal portion forms a > -shaped,

darkly stained strip, with the concavity facing the amnion. The concavity of the > is occupied by the ectodermal thickening of the amnion. The distal part of the cord, also darkly stained, is directly continuous cranially with the large group of round cells noted in the previous section. The outgrowth from the primitive streak immediately caudal to Hensen's node is still present but it is much smaller. The node itself is larger, as the sections at this level are close to the median plane. The roof of the yolk-sac cranial to the node is formed by a number of low columnar cells, which are not so obviously in continuity with the node owing to the presence of a shrinkage interval. Their



Text-fig. 23. Section 19·10. ×200.

nuclei are large and darkly stained, resembling in every way the nuclei in the node. They are separated from the shield ectoderm by a group of three slightly elongated mesoderm cells which also appear to be derived from the node. At the cranial end the precocious coelomic cavity is again present and well formed. Caudal to it the entoderm is again adherent to the shield ectoderm. In this situation it is slightly thickened and consists of a number of large, vesicular cells with round nuclei.

19·10 (Text-fig. 23). Most of the connecting stalk is still occupied by the remains of the cloacal membrane, but in its caudal part there is a greatly increased number of mesenchymal cells. Hensen's node is cut approximately

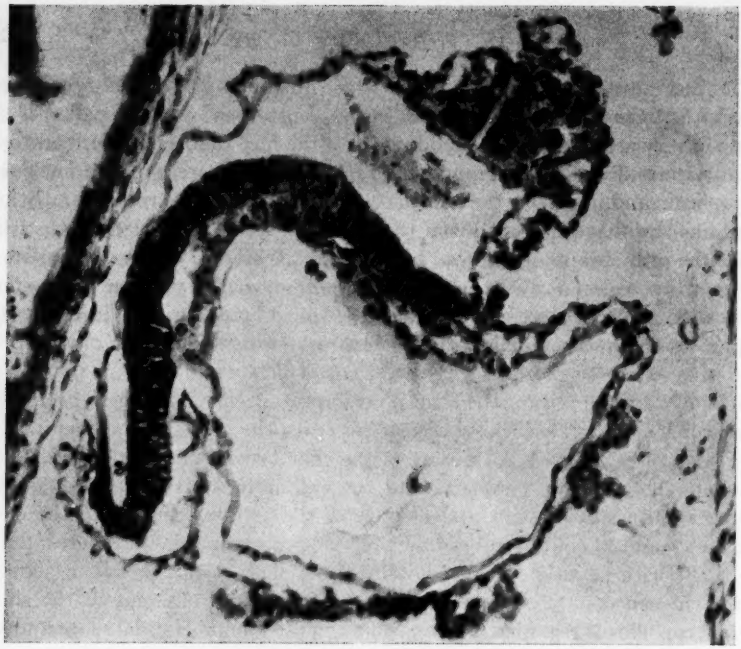
in its median plane. It involves the whole thickness of the embryonic area and no entodermal cells are present on its ventral surface. The pallor of the node is very striking. This is due in part to the fact that its nuclei tend to be grouped into oblique, overlapping rows,[1] leaving wide areas of non-nucleated cytoplasm, and in part to the fact that the cytoplasm stains rather faintly and contains shrinkage spaces. From the cranial surface of the deep part of the node the primordium of the head process extends cranially. It now consists of five narrow, columnar cells which form the roof of the yolk-sac and extend almost to the basement membrane of the shield ectoderm. A shrinkage gap separates the most cranial of these cells from two smaller cells, obviously of the same character, although they are not typically columnar in shape. The nuclei of all these cells of the head process stain darkly, showing a prominent nucleolus, and are identical in appearance with the nuclei in the deep part of the node. From the caudal surface of the node typical mesodermal cells grow caudally between the ectoderm and the entoderm. A single, desquamated entodermal cell is present in the cavity of the yolk-sac ventral to the node. At the cranial end of the embryo the precocious coelomic cavity is here reduced to a slit with slightly thickened walls. Caudal to it the entoderm shows a large thickened patch of vesicular cells and is again adherent to the shield ectoderm. This is regarded as the primordium of the prochordal plate and will be referred to under that name.

19·11 (Text-fig. 24). The last traces of the cloacal membrane are visible in the connecting stalk. Hensen's node and the head process are readily recognizable. From the caudal surface of the node mesodermal cells are extending caudally between the ectoderm and the entoderm. Two desquamated entodermal cells lie free in the yolk-sac (below the caudal part of the node in the figure). At the cranial end of the embryo the coelomic cavity is obscured, but caudal to it the prochordal plate is a conspicuous feature.

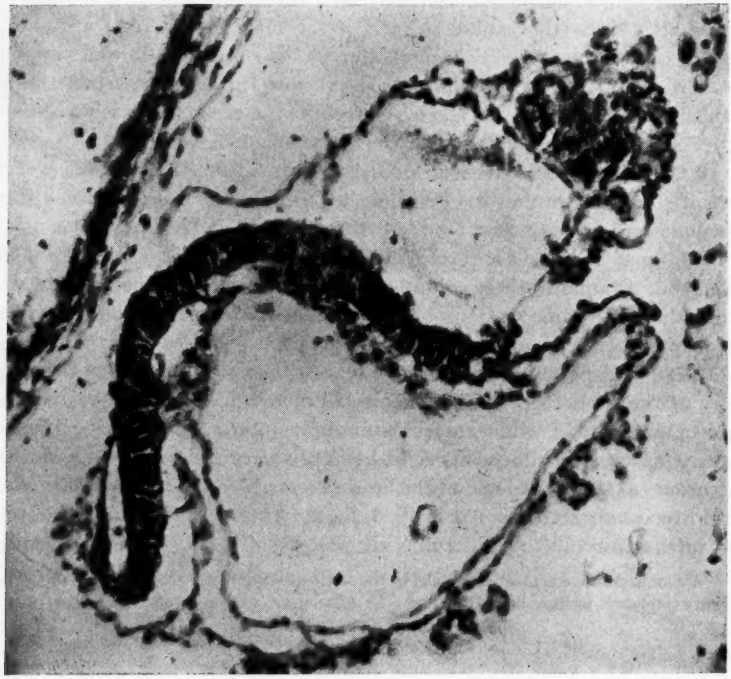
19·11 (Pl. 2, fig. 1). Hensen's node is shown $\times 950$. The wide areas of non-nucleated cytoplasm and the crowding together of the nuclei are well shown. The node contains a mitotic figure near its centre. The head process consists of five columnar cells, and a mitotic figure is present on its ventral surface at its nodal end. Cranial to these columnar cells there are three smaller cells, whose nuclei show the same staining affinities as the nuclei of the node; they probably represent the headward end of the process.

19·12 (Text-fig. 25). Caudally the tear in the shield has led to the sagging away of the connecting stalk. The cloacal membrane has disappeared, and a mass of mesodermal cells occupies the site of its more ventral portion. Hensen's node, which is divided near its right border, is very conspicuous. A shrinkage gap occurs at the cranial end of the head process. The structure of the node and head process are shown $\times 950$ in Pl. 2, fig. 2. At the cranial end of the embryo the intra-embryonic mesoderm is in process of cavity formation, and the

[1] A similar arrangement of the nuclei in the ferret embryo can be seen in some of the figures recently published by Hamilton (1937).



Text-fig. 24. Section 19·11. ×200.



Text-fig. 25. Section 19·12. ×200.

ventral cells show the "syncytial ribbon" appearance already noted in the roof of the amnion and the cranial wall of the yolk-sac. The prochordal plate stands out prominently; its detailed structure is shown ×950 in Pl. 3, fig. 1.

19·12 (Pl. 2, fig. 2). The nuclei in Hensen's node are arranged in two oblique, overlapping rows, directed cranially and ventrally. They are not remarkable for their size. The head process consists of three or four columnar cells which occupy nearly the whole space between the shield ectoderm and the roof of the yolk-sac. There is obvious tissue continuity between the caudal end of the process and Hensen's node.

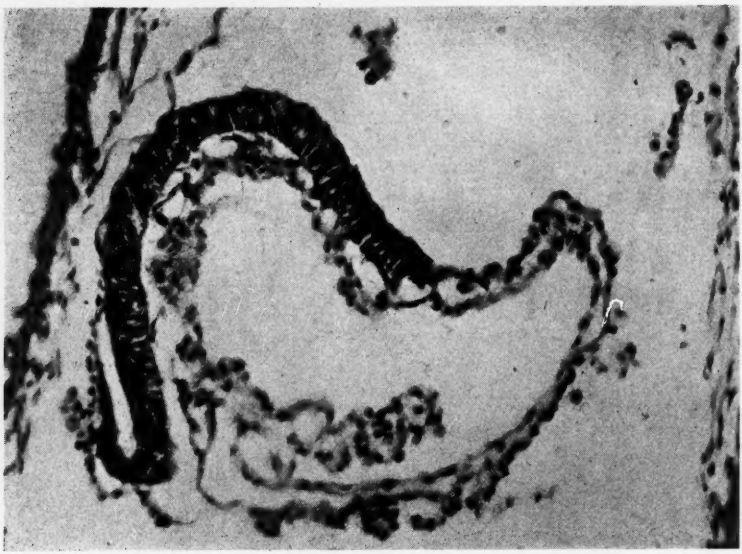
Text-fig. 26. Section 20·1. ×200.

19·12 (Pl. 3, fig. 1). The prochordal plate consists of about ten large, vesicular, entodermal cells. Their nuclei do not stain darkly and appear oval in this section. The plate is adherent to the basement membrane of the shield ectoderm over a considerable area.

20·1 (Text-fig. 26). The mass of mesodermal cells seen in the connecting stalk in the preceding section is again prominent. Only the dorsal part of Hensen's node is cut, as the section passes close to its right border. Cranial to it the interval between the ectoderm and the yolk-sac is occupied for 0·03 mm. by cells which may be regarded as forming the right edge of the head process.

At the cranial end of the embryo the intra-embryonic mesoderm contains a very distinct cavity, which is less extensive in the cranio-caudal axis than it is in some of the preceding sections. Its walls are beautifully fixed and show the "syncytial ribbon" appearance. Caudal to it the prochordal plate is a conspicuous feature.

20·1 (Pl. 3, fig. 2). The prochordal plate is only adherent to the shield ectoderm at one point. It is of approximately the same thickness as the shield and comprises fourteen or fifteen large vesicular cells with spherical or oval nuclei, which display a prominent nucleolus and a fine chromatin network. A fine coagulum extends into the yolk-sac from the ventral surface of the plate.



Text-fig. 27. Section 20·2. ×200.

20·2 (Text-fig. 27). Unfortunately in this section the connecting stalk has floated away from the embryo and has become so wrinkled that its structure cannot be examined. The last trace of Hensen's node is seen as a pallid area in the most dorsal part of the shield. Cranial to it mesodermal cells fill up nearly the whole interval between the shield ectoderm and the yolk-sac, the roof of which is again entodermal. Clearly these cells lie on the right side of the head process and may belong to or may be derivatives from it. At the cranial end of the embryo the precocious coelomic formation appears for the last time. The prochordal plate is no longer adherent to the ectoderm. In this and in the preceding section there is an extensive wrinkling of the wall of the yolk-sac where the roof becomes continuous with the cranial wall.

20·3 (Text-fig. 28). The sections have now reached the right margin of the caudal end of the shield. The angled recess at the dorsi-caudal end of the amniotic cavity is being partially filled up from its right side, as the section is shaving obliquely through the amniotic covering of the connecting stalk. The recess disappears in the ensuing sections. The prochordal plate is easily identified, but some mesoderm cells now intervene between it and the ectoderm. Elsewhere a false appearance of entodermal thickening is presented in the roof

Text-fig. 28. Section 20·3. ×200.

of the yolk-sac. This is due to the fact that the sections are now rapidly approaching the right wall of the sac, which is cut obliquely in several places. The wrinkling of the cranial wall at its junction with the roof gives rise to a very exuberant infolding in this section. The succeeding sections pass through the embryo with increasing degrees of obliquity and are entirely to the right of the median plane. As the median plane structures of the shield are no longer divided, only selected sections from the rest of the series will be figured.

20·8 (Text-fig. 29). This section shows a substantial shaving through the right wall of the yolk-sac, which appears as an island of solid entoderm within

the concavity of the shield. The connecting stalk, now approaching the chorion, is looser in texture and shows the presence of a number of vascular spaces.

20·10 (Text-fig. 30). This section, after dividing the shield, passes through the interval between its right sloping surface and the right wall of the yolk-sac. The interior of the concavity of the shield is occupied by the branching, interconnected cells of the intra-embryonic mesoderm, and the general continuity

Text-fig. 29. Section 20·8. ×200.

of the open meshwork of the mesoderm is well demonstrated. In many places the shield ectoderm appears to be giving origin to mesoderm cells, but, owing to the obliquity of the section, too much stress must not be laid upon this appearance. A well-formed vascular space, lined by flattened elongated cells, is seen in the connecting stalk (lower and left part in Text-fig. 34). Other, less convincing, spaces are also present.

From this point onwards, owing to the curvature of the shield and the obliquity of the section plane, the sections through the shield ectoderm become more and more nearly tangential.

21·4 (Text-fig. 31). This section cuts through the right surface of the shield tangentially so that it appears as a solid mass of ectoderm. The connecting stalk is very loose in texture and has almost reached the chorion.

21·10 (Text-fig. 32). This section shaves through the right surface of the upturned rim of the amnion to the right of the right border of the shield. The connecting stalk is now in continuity with the primary mesenchyme of the chorion. Both the stalk and the cavity of the amnion can be recognized in the succeeding sections.

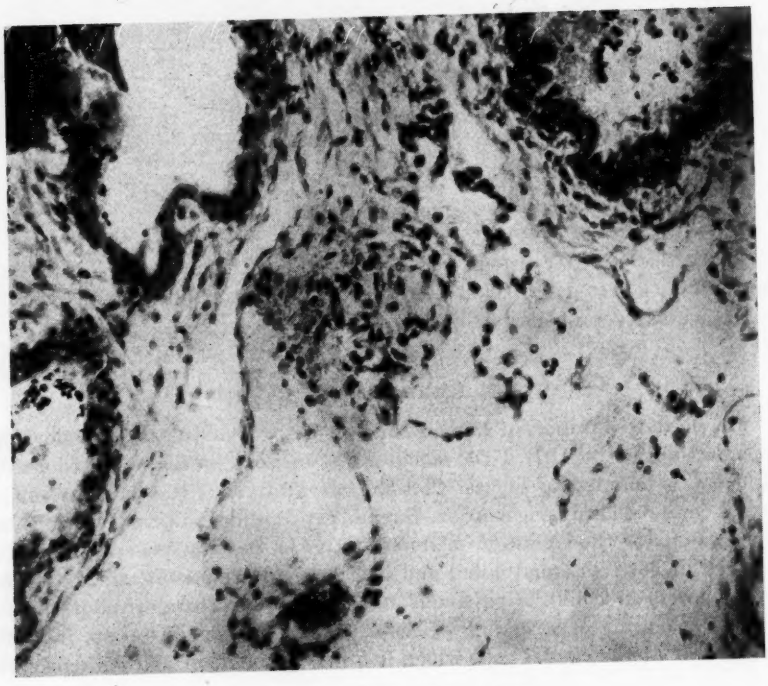
Text-fig. 30. Section 20·10. × 200.

Now that the reconstruction model and all the important individual sections have been described, it is possible to discuss the salient features of the embryo and the problems arising from them.

THE EMBRYONIC SHIELD

In most well-preserved human embryos at or near the stage of development exhibited by H.R. 1 the shield is flat or very gently convex dorsally, and one is tempted to suggest that the curvature of H.R. 1 is the result of slight compression during fixation. Such an explanation, however, is insufficient to account for the condition in its entirety. In the first place the shield is convex in both its longitudinal and its transverse diameters, and no degree of compression could bring about this double curvature without causing excessive tearing or wrinkling of the surface of the shield. Apart from the small tear at the caudal end, which is quite incapable of accounting for the

Text-fig. 31. Section 21·4. ×200.



Text-fig. 32. Section 21·10. ×200.

condition, the shield is intact and shows no sign of wrinkling. In the second place the yolk-sac shows all the appearances that might be expected if the curvature were the result of a growth process. Its dome-shaped roof is everywhere adapted to the curve of the shield and shows no sign of wrinkling or tearing.

On these two grounds I believe that the curvature of the shield is the result of abnormal growth, though it is probable that it has been exaggerated to some extent during the process of fixation. Attention has already been drawn to the fact that the embryo was situated in the deepest part of the chorionic cavity, which in this case is the narrowest part of the space. It is not unlikely that the confinement of the embryo in this restricted space may have helped to account for its abnormal shape.

One would not, however, be justified on this account in discounting entirely the importance of the evidence which this embryo offers with regard to other developmental problems, for abnormalities of shape are by no means uncommon. The shield of the Strahl-Beneke embryo is concave dorsally, while in the Bi. 1, the T.F. and the Heuser (1932) embryos it is convex like the shield of H.R. 1, but, nevertheless, all these embryos have provided important contributions to our knowledge of the normal development of the human embryo in its early stages.

The shield ectoderm consists of high columnar cells, arranged usually in three, though occasionally in four and sometimes in two, overlapping rows. The cells, for the most part, are set at right angles to the free surface, but in many places their long axes are oblique (Pl. 3, fig. 1), a fact which may be regarded as confirming the view that the shield curvature is the result of growth processes. At its caudal end the shield ectoderm is abruptly reduced to a single layer of rather flattened cells over a length of 0·06 mm. This reduction in thickness is present in both the Strahl-Beneke and the Hugo embryos, and in both it extends as far caudally as the cloacal membrane. Further reference will be made to this feature when the primitive streak is discussed.

The cytoplasm of the cells of the ectoderm stains darkly, and the nuclei, which are elongated or oval for the most part, show a rich chromatin network and a prominent nucleolus. In the surface layer numerous cells occur with enlarged, almost spherical nuclei, the cytoplasm of which is less darkly stained. These may possibly be cells which are about to undergo mitosis. In most of the sections mitotic figures are present, the cells concerned being, with few exceptions, situated in the most superficial layer. Only one was found in the caudal part of the shield, but three were observed in the depths of Hensen's node. This distribution of mitotic figures agrees well with the condition found in both the Hugo and the Strahl-Beneke embryos, and may be regarded as indicative of rapid increase in size of the pre-nodal part of the shield. It is clear also that enlargement of the shield at this stage is brought about by the division of the cells of the superficial layer.

Intracellular darkly staining droplets are a very striking feature in most of the sections (Pl. 2, fig. 1). They vary considerably in size and are found at all depths from the surface, being most numerous in the cranial two-thirds of the shield and in the region of Hensen's node. In places the droplets run together, forming crescentic figures, and they are usually surrounded by a small area of lightly stained cytoplasm. Droplets, identical in appearance, are found within the cavity of the amnion (Text-fig. 24), and their presence there in considerable quantity suggests that they may be of the nature of an excretion. They have been observed by Florian in the Embryo Fetzer (1929).

No signs of degenerative changes were seen in the superficial cells of the shield. Von Möllendorff (1921 *a*) described such changes in the Ovum O.P. and regarded them as normal. Stieve (1926) opposed his interpretation, and the evidence available from H.R. 1 strongly supports his view.

Except in the regions of Hensen's node and the primitive streak, the deep surface of the shield ectoderm is sharply demarcated, and the appearance is strongly suggestive of the presence of a basement membrane. Graf v. Spee (1896) was the first to describe the existence of such a membrane, and it has been identified more recently by von Möllendorff (1921 *a*), Rossenbeck (1923), Ramsey (1937), and others, so that it may be regarded as a normal structure. In H.R. 1 the shield shows the presence of many fine, obliquely vertical slits which are the result of shrinkage during fixation. These slits are visible in many of the figures, and it is to be noted that, although in some places they reach the basement membrane, they do not break through it. In von Möllendorff's Ovum O.P. the basement membrane was partially detached, a fact which Stieve (1926) regards as indicative of poor fixation. In no situation is the basement membrane detached in H.R. 1.

At the cranial border of the shield, and along each lateral border, the transition from the shield ectoderm to the typical, flattened epithelium of the amnion is gradual. As a result the shield appears to have a turned-up rim everywhere except at its caudal end. This rim is regarded as part of the amnion and has been excluded in estimating the measurements of the shield. It bears no relation to the entoderm of the yolk-sac, and the primary mesoderm passes continuously from its outer surface on to the cranial and lateral walls of the sac. A similar rim is present along the lateral margins of the Fetzer embryo (Florian, 1933), and in the Strahl-Beneke the transition from shield to amnion at the cranial end is so gradual that it is difficult to determine the precise cranial limit of the embryonic area.

Streeter (1936) has recently put forward the view that the first and most fundamental differentiation in the morula mass separates the cells which take part in the formation of the embryo itself from those which form only extra-embryonic structures. Amongst the latter he includes the cells of the amnion, and as evidence in favour of this view he cites the abrupt character of the transition from the shield ectoderm to the ectoderm of the amnion. This view will be considered later in connexion with the yolk-sac, but from a study of

the embryos cited it is clear that the transition from shield ectoderm to amniotic ectoderm may be either gradual or abrupt, and that its character cannot be adduced as evidence in support of Streeter's view.

THE PRIMITIVE STREAK

The primitive streak, including Hensen's node, has been identified in sections 19·4–20·2. It measures 0·115 mm. in length and its caudal end falls short of the caudal end of the shield by 0·09 mm.

The cranial half, or rather more, of the streak can readily be distinguished from its caudal half. It stands out as a relatively pale area in the darkly stained shield (Pl. 2, fig. 2) and shows features which indicate that it may justly be regarded as an early stage of Hensen's node. At the cranial end of the streak the sharp contour of the deep surface of the shield ectoderm ceases abruptly, and there is tissue continuity through the whole thickness of the embryonic area, the entoderm being absent from the ventral surface of the streak. The structure of the node has already been described and reasons have been given for its characteristic pallor (p. 23). In older embryos, such as the Manchester embryo (Hill & Florian, 1935), the contrast between the pallor of the node and the dark staining of the adjoining primitive streak is sometimes very striking. No such contrast, however, is present in the figures of the Embryo Hugo or Strahl-Beneke, and a very similar pallid area in the Fetzer embryo is regarded by Florian as the primitive streak itself prior to node formation. No surface elevation marks the position of the node, but, although Stieve lays stress on its presence as a means of identification, it is clear from Embryo Bi. 24 that a typical node may be present with little or no surface elevation. In older embryos, the surface elevation is more constant. In identifying the pallid area in H.R. 1 as Hensen's node, I have been more influenced by the fact that it is giving rise to the primordium of the head process.

The primitive streak proper is very short, measuring only 0·055 mm. long. It is in an early stage of differentiation and does not yet extend to the cloacal membrane, being separated from it by a zone of undifferentiated ectoderm, 0·09 mm. long. The constituent cells of the streak are wider than those of the rest of the shield ectoderm and have large oval nuclei. They are packed closely together and few shrinkage slits, such as have been described on p. 32, can be seen. The cytoplasm of the cells stains darkly and no difficulty is experienced in determining where the streak ends and Hensen's node begins. At its caudal end the deep surface of the streak gives origin to a group of cells (Text-fig. 18) which stream into the connecting stalk. These cells are, I believe, identical with those described by Stieve as forming the "Sichel-knoten" in the Embryo Hugo and by Florian as the "end node" in the Strahl-Beneke embryo. They must be regarded as characteristic of the early stages of the primitive streak, but no light has yet been thrown on their significance.

The small mass of large ectodermal cells which springs from the cranial

end of the streak proper (Text-fig. 20) is difficult to interpret, for the cells do not appear to be mesodermal, as they retain their ectodermal characters unchanged. The only approximately similar appearance which I have been able to find in the literature is in the Meyer (1924) embryo, where it was identified—wrongly according to Florian (1928)—as the head process. If the two structures are actually identical, Meyer's interpretation must be erroneous, for in H.R. 1 the mass lies caudal to Hensen's node.

The ventral surface of the streak proper is finely irregular and shows no trace of the presence of a basement membrane. Ventral to it the entodermal roof of the yolk-sac is intact.

Owing to the section plane it is impossible to be certain whether the edges of the streak proper are actively giving rise to intra-embryonic mesoderm or not. The subject will be dealt with later, when the intra-embryonic mesoderm is considered.

THE HEAD PROCESS

A short mass of cells, broader at its caudal end than at its cranial end, has been identified as the primordium of the head process in H.R. 1. The mass is clearly in continuity with the cranial end of Hensen's node and its nuclei are identical, both in staining reactions and in appearance, with the nuclei of the node. It is not more than 0·04 mm. long and is therefore in an early stage of development. Except near its cranial end, which is difficult to determine precisely, the cells of the process are columnar and present a picture (Text-fig. 23) which suggests that they may in reality be entodermal. That possibility, however, is excluded by the continuity of the process with Hensen's node and by the character of its nuclei. Further, within the cavity of the yolk-sac in this region there are five desquamated entodermal cells (one each in 19·7, 19·8 and 19·10 and two in 19·11) in addition to some detritus in 19·12 which may represent the remains of one or two entodermal cells. It is suggested that these are some of the cells which originally formed the roof of the yolk-sac in this situation and which have been replaced by the cells of the head process. In this connexion it should be observed that in the Embryo Fetzer, which shows no sign of a head process, the cells in the median plane of the yolk-sac roof immediately cranial to the primitive streak are vesicular in character.

Apart from the Embryo Meyer, which has already been discussed and excluded, the H.Schm. 10, the Hugo and the Bi. 24 embryos are the youngest which show the presence of an undoubted head process. In H.Schm. 10 a head process, 0·1 mm. long, is already present and in one section it contains a space, lined dorsally by high columnar epithelium and ventrally by low columnar epithelium, which is identified by Grosser (1931*b*) as Lieberkühn's canal. In the Hugo embryo the head process is 0·09 mm. long and consists of an irregularly defined mass of mesodermal cells, more compact at its caudal end than at its cranial end. The cells of which it consists are in continuity with the intra-embryonic mesoderm on each side and with Hensen's node caudally;

they are fusiform or rounded in shape for the most part, but nowhere are they columnar. In no human embryo yet described, with the exception of Grosser's H.Schm. 10, have any columnar cells been observed in the head process. According to Stieve the head process of the Embryo Hugo has no entoderm on its ventral surface, but Florian believes that the entodermal roof of the yolk-sac is intact. I am satisfied that the structure in question in H.R. 1 is a derivative of Hensen's node, but it will be necessary to wait until other embryos of the same stage are available before one can be certain that it is the normal, early condition of the head process.

The cloacal membrane will be described with the entoderm (p. 38).

THE YOLK-SAC

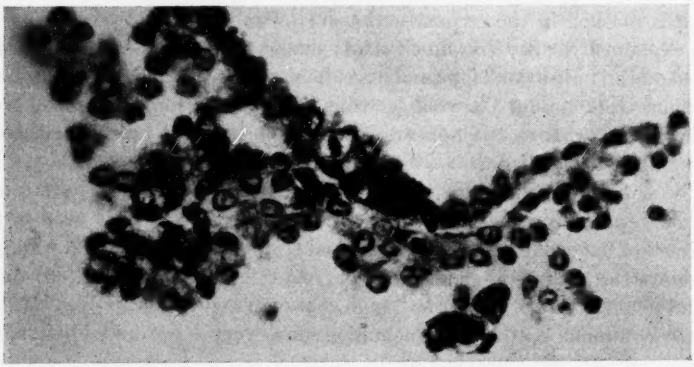
Attention has already been drawn to the small size of the yolk-sac and this feature can be appreciated better from a consideration of Table 1. The reasons why it is masked in the reconstruction model and in the actual sections have been explained earlier. Too much stress should not be laid on the size of the yolk-sac as an indication of normal development, for although there is naturally a distinct tendency for the yolk-sac to enlarge proportionately to the growth of the shield prior to the appearance of the head- and tail-folds, there are many notable exceptions.

The entoderm lining the yolk-sac is remarkably varied in its structure. The roof, apart from the region of the prochordal plate and the head process, is formed of flattened cells and low, cubical epithelium. The ventral wall of the sac shows the widest range of variation. At one point (Text-fig. 33) there is a small patch of five or six typical, high columnar cells. A large area is covered by low columnar epithelium, including some very large and obviously degenerating cells with spherical nuclei, some of which are hydropic and others intensely pycnotic. Low columnar epithelium forms the ventral wall of the caudal diverticulum in the neighbourhood of the origin of the allantoic cord (Text-fig. 9). Elsewhere the cells are elongated and slightly flattened. In patches these cells are almost endothelial in their appearance; often they form what can only be termed a "syncytial ribbon". That the last two varieties are different forms of the same type is shown by their repeated occurrence in immediately adjoining areas, and by the fact that where the "endothelial" type occurs the cytoplasm is obviously coagulated and shrunken. The same two types of epithelium are found in adjoining patches in the Strahl-Beneke embryo, and are present, though not so conspicuous in the Embryo Hugo. The "syncytial ribbon" type appears to be better fixed than the "endothelial" type.

In all embryos the ventral wall of the yolk-sac shows the greatest range of variation as regards the character of the cells.

In view of the varieties of epithelium to which the intra-embryonic entoderm gives origin in the course of development, the variations noted in H.R. 1 are not altogether surprising. Nevertheless, it must be stated that the cells in

the roof show less variation than the other walls, and this may prove to be of importance in connexion with (1) the view advanced by Corning (1925) and supported by Stieve (1936) on the evidence of the findings in the Embryo Werner; and (2) the view recently advocated by Streeter (1936). Corning's theory that the yolk-sac cavity is originally part of the magma cavity outlined in primary mesoderm and that the entoderm of the roof, which is derived from the inner cell mass, gradually spreads over the interior of the cavity, is neither confirmed nor refuted by the evidence from H.R. 1. The possession of an obvious variety of potencies in the cells of the ventral wall of the yolk-sac might, on the other hand, be regarded as evidence in favour of Streeter's view (already stated), in accordance with which differences in the characters of the roof and the other walls of the yolk-sac might be anticipated. It must, however, be emphasized that at this stage the potencies of the cells concerned are



Text-fig. 33. A portion of the wall of the yolk-sac. × 392.

apparently not yet materially reduced and the exhibition of different tendencies in different areas does not of itself necessarily imply differences in developmental origin. I regard the fact that the "syncytial ribbons" are found (1) in the roof of the amnion, (2) in the walls of the precocious coelomic cavity (see later), and (3) in the wall of the yolk-sac, as evidence that the differentiation of the ecto- ento- and mesoderm has not yet affected seriously the potencies of the cells concerned.

THE ALLANTOIC CORD

The condition of the allantoic representative in the H.R. 1 calls for detailed consideration. It is a solid cord of entoderm which—due allowance being made for the kink in the connecting stalk—arises in the median plane from the apex of a funnel-shaped diverticulum from the caudal wall of the yolk-sac. It passes at once into the connecting stalk and measures 0·135 mm. from base to tip. At its distal end it takes part in the formation of the cloacal membrane over an area 0·1 mm. long.

The early history of the allantois in the human embryo is still very obscure. In the Embryo Bi. 24 (Florian, 1933), which is at a slightly later stage of development than the Hugo embryo, an allantoic canal is present, measures 0·13 mm. in length, and takes a large share in the formation of the cloacal membrane. In all older embryos it is present as a patent, tubular diverticulum. It measures 0·19 mm. in the Manchester embryo (Hill & Florian, 1935), 0·14 mm. in the Thompson-Brash (1923), and 0·21 in the Peh. 1-Hochstetter (Rossenbeck, 1923). Its relationship to the cloacal membrane in these older embryos is variable. In the Manchester, the Dobbin (Hill & Florian, 1931) and the Thompson-Brash it takes no part in the formation of the membrane, whereas in the Peh. 1-Hochstetter and Grosser's embryos Kl. 3 (1913) and Wa. 17 (1931) it forms a large part of it.

In the Ingalls embryo (1918) there is a small area of ecto-entodermal connexion involving the allantois and separated by a gap from the cloacal membrane proper.

In the Embryo Hugo, according to Stieve's (1926) description, "ist kein deutlicher Allantoisgang oder eine Allantoisbucht vorhanden". A very small recess in the dorsal part of the caudal wall of the yolk-sac is tentatively regarded as the allantoic representative.

In embryos at an earlier stage of development than the H.R. 1 the condition of the allantoic representative has given rise to considerable discussion. Grosser (1913) identified an allantoic diverticulum in the Embryo Peters, and von Möllendorff originally described a large diverticulum of the yolk-sac in the Embryo O.P. as the allantois, but in both these embryos it is now generally agreed that no allantoic representative is present. In the Embryo W.O., which is slightly older than the O.P., a solid mass of thickened entoderm projects dorsally from the roof of the yolk-sac at the caudal end of the embryonic area. Von Möllendorff (1925) regards this as the primordium of the allantois, and put forward the view that in the human embryo the allantois arises as a solid outgrowth which acquires a lumen later. Florian (1928) opposes this view and regards the entodermal outgrowth as the primordium of the cloacal membrane, which he has identified in the Embryos Fetzer and Bi. 1. In the figures already published of Bi. 1 (the full description has not yet appeared) the allantois is represented by a solid entodermal outgrowth with a small central cavity, which projects from the caudal wall of the yolk-sac and fuses with the ectoderm over a small area. Florian, however, is now of opinion that this structure is not the definitive allantois but is destined to become incorporated in the yolk-sac and to be replaced by the true allantois at a later stage. In the Embryo Fetzer no trace of any allantoic representative is present.

The condition in the Strahl-Beneke embryo furnishes a close parallel to the condition in H.R. 1. The yolk-sac, which is slightly distorted, gives off an irregularly shaped recess at the junction of its roof with its caudal wall. This recess may in reality be due to the distortion, but it seems to represent the

funnel-shaped caudal recess present in H.R. 1. From its summit a solid entodermal cord passes into the connecting stalk and comes into continuity near its distal end with the ectoderm of the amnion. It measures 0·09 mm. long. In the Embryo T.F. the allantois is, doubtfully in my opinion, represented by a small diverticulum from the dorsal end of the caudal wall of the yolk-sac, so that the condition is not dissimilar to the condition of the Embryo Hugo. In the Embryo Meyer a funnel-shaped diverticulum projects dorsally from the caudal end of the yolk-sac roof into the connecting stalk. The end of the diverticulum is formed by a short, solid mass of entoderm, which may possibly be more extensive than is represented in Meyer's figures. Florian (1929) is of opinion that a cloacal membrane is probably present in connexion with the allantoic outgrowth, which measures not less than 0·12 mm. in length. In Grosser's Embryo H.Schm. 10 (1931) the allantois is represented by a tubular diverticulum with relatively thick walls and a very narrow lumen. It is 0·05 mm. long and in the neighbourhood of its solid tip its epithelium is fused with the amniotic covering of the connecting stalk.

From this evidence it is possible to draw some tentative conclusions with reference to the early development of the allantois. The embryos W.O., Bi. 1, Strahl-Beneke, H.R. 1, Meyer and H.Schm. 10 form a series which suggest that the primordium of the allantois is a solid entodermal cord which establishes continuity with the ectoderm of the amnion covering the connecting stalk at an early stage. It acquires its lumen later, probably as an extension of the yolk-sac cavity into the cord. On the other hand, the conditions found in the Embryo Hugo and the Embryo T.F. make it clear that the actual time of appearance of the allantoic representative is subject to considerable variation. If the small diverticula from the yolk-sac in both embryos are accepted as representing the allantois, only two alternative explanations are possible. (1) The allantoic representative appears as a solid cord which soon becomes atrophied and disappears, its proximal end forming the site of the hollow allantoic canal. (2) The allantois may develop either as a solid entodermal rod, or as a small, hollow diverticulum which rapidly enlarges and grows into the connecting stalk. On the whole it seems more probable that the development of the allantoic primordium has been delayed in both cases, and that the identification of the small diverticula as allantoic representatives is not justified. The question, however, is complicated by the relationships exhibited by the cloacal membrane.

THE CLOACAL MEMBRANE

This remarkable structure has been very fully investigated by Florian, who has succeeded in identifying it in embryos as early as the Fetzer and the Bi. 1. Further he believes that it is probably present in the Embryo W.O. It was therefore confidently anticipated that a cloacal membrane would be present in the H.R. 1, but no connexion could be observed between the roof of the yolk-sac and the shield ectoderm. Prof. Florian, to whom I had sent

photographs of the sections, identified it caudal to the shield, and further examination of the sections showed that his identification was justified. In the H.R. 1 (Text-figs. 19 and 21) the cloacal membrane is associated with the terminal 0·1 mm. of the allantoic cord and extends between it and the amniotic covering of the connecting stalk immediately caudal to the shield. It is cut obliquely but its identification is unequivocal in sections 19·7 and 19·8, though it is not quite so convincing in sections 19·6, 19·9 and 19·10. The ectodermal cells of the most caudal part of the membrane (upper part in the figures) were mistaken at first for a large blood island, but further examination demonstrated their continuity with the cells of the allantoic cord and with the cells lying cranial to them (below, in the figures). These cells contain a few of the darkly staining droplets which have been mentioned in connexion with the shield ectoderm. It is important to observe that in the Strahl-Beneke embryo also the ectoderm concerned in the formation of the cloacal membrane is amniotic. On the other hand, both in the Bi. 1 and the Fetzer embryos Florian has identified the membrane within the limits of the shield and in Embryo Bi. 24 it is situated partly in the shield and partly in the connecting stalk. In older embryos it is situated either wholly within the shield (Thompson-Brash, Manchester, Dobbin, etc.) or it may involve both the caudal end of the shield and the adjoining amniotic ectoderm of the connecting stalk (Peh. 1-Hochstetter, Sternberg (1927), Grosser's Kl. 3, etc.).

The question at once arises as to whether the cloacal membrane in H.R. 1 and in the Strahl-Beneke embryo is identical with the structure described by Florian in the Fetzer embryo (1930) and in the Hugo embryo. In both H.R. 1 and the Strahl-Beneke embryo the cloacal membrane is outside the shield area and involves the allantoic cord only. In the Fetzer embryo it lies within the limits of the shield and involves the roof of the yolk-sac (the allantois is not yet present). It may be, as Wyburn (1937) appears to believe, that the cloacal membrane in the Fetzer embryo indicates the site at which the allantois will subsequently develop, but Florian in his Figs. 1*a* and 1*b* places it definitely within the area of the shield. If the two structures are identical it follows that, in order to account for the conditions found in the older embryos, the cloacal membrane may appear either in the connecting stalk and later extend cranially on to the shield, or in the shield and later extend caudally on to the stalk. That such a variability in the origin and growth of the cloacal membrane should occur is extremely improbable, and one is forced to the conclusion that if Florian's identification of the membrane in the Embryo Fetzer is justified, the two structures cannot be identical. It would then be necessary, in order to explain the ecto-entodermal fusion in the Strahl-Beneke and the H.R. 1, to fall back on the possible occurrence of a "canalis amnio-allantoideus". This structure was described by Schauinsland (1902) in Lacerta and was called in by Ingalls to explain a very small area of amnio-allantoic fusion (1918). Schauinsland's description for Lacerta is far from convincing and there is no evidence available to justify the suggestion that the rudiments of such a

structure are normally present in the human embryo, and the fact that amnio-allantoic fusion is present in the Strahl-Beneke, the H.R. 1 and the H.Schm. 10 embryos is sufficient to show that the fusion is a normal feature. In discussing Grosser's H.Schm. 10, Florian (1933) makes it clear that he regards ecto-entodermal fusion and not contact as the criterion for the recognition of the cloacal membrane. He rejects an area of contact at the caudal end of the primitive streak within the shield area and accepts the amnio-allantoic fusion in the connecting stalk as the cloacal membrane. In this way he brings the H.Schm. 10 into alignment with the Strahl-Beneke and the H.R. 1.

The whole question, however, bristles with difficulties and can only be solved when further material has become available. Meantime, I incline to the view that the cloacal membrane develops later than the allantois and in close association with it, and at a later period extends into the caudal end of the shield. This is a modification of the view recently put forward by Wyburn (1937) who, however, had access to no material younger than the Embryo MacIntyre 1, which has well-developed head- and tail-folds and measures 1·4 mm.

THE PROCHORDAL PLATE

The patch of thickened entoderm which forms such a conspicuous feature in the roof of the yolk-sac in sections 19·9–20·3 has already been identified as the primordium of the prochordal plate. It measures 0·075 mm. long × 0·05 mm. wide and its cranial limit lies 0·12 mm. from the cranial border of the shield. Except towards its right margin it is connected to the basement membrane of the shield ectoderm over an area which varies in extent from section to section. It is certain that the connexion was both real and extensive, probably considerably greater than is represented in the median section of the model (Text-fig. 2). In most of the sections in which it is cut the plate consists of from ten to twelve large, vesicular cells, with large, round or oval nuclei and with only suggestions of cell boundaries. The cytoplasm is lightly staining and contains many vacuoles. The ventral surface of the plate is covered with the fine coagulum content of the yolk-sac, and it is uncertain whether or not the plate is continuous at its caudal end with the cranial end of the head process, although the continuity, if it exists, is exceedingly tenuous.

Florian (1933) has described and figured a patch of similar cells in the roof of the yolk-sac in the Embryo Fetzer, but in this case the cells form a single layer and lie immediately cranial to the primitive streak. A corresponding patch of thickened entoderm is present in the Strahl-Beneke embryo and its position and the characters of its constituent cells indicate quite clearly that we are dealing here with the same structure as is present in H.R. 1. In the Embryo Hugo, on the other hand, Stieve (1926) makes no mention of the existence of any corresponding structure although his Figs. 12 and 13 are rather suggestive, and in the Embryo H.Schm. 10 Grosser (1931*b*) says "Eine Prächordalplatte lässt sich nicht mit Sicherheit nachweisen".

In the Bi. 24 and older embryos the prochordal plate is a constant structure.

The prochordal plate in the Strahl-Beneke embryo and in H.R. 1 might conceivably have another interpretation. The vesicular character of the cells concerned might be regarded as a degenerative change prior to desquamation to provide space for the growing head process. On the other hand, the nuclei are healthy and active in appearance, and desquamation does not necessitate a prior thickening.

Attention should be drawn to the adhesion of the plate to the basement membrane of the shield ectoderm, and to the relation existing between the plate and the precocious coelomic cavity (see later). Both point to the possibility of a relationship between the prochordal plate and the buccopharyngeal membrane.

CONTENTS

The yolk-sac contains in places a small amount of very lightly staining and foam-like coagulum which, although situated centrally for the most part, is also present in many sections in contact with the roof and the caudal wall. It is seen in Pl. 3, fig. 1 in close relation to the prochordal plate. The presence of such a coagulum in the yolk-sac has been noted by many observers and may indicate secretory activity of the entoderm, or it may be the result of post-mortem changes. In addition the yolk-sac contains a few desquamated entodermal cells, especially in the region of the head process as already stated.

ENTODERM CYSTS

A careful search was made for isolated entodermal cysts in the chorionic cavity, although the fact that the specimen had been reblocked after the cavity had been opened implied that a negative result would not necessarily mean that none had been present originally.

In 7·2–9·2 a patch of undoubted entodermal cells was discovered which had become entangled in a film of coagulum. It comprises four to ten cells in each section. The nuclei are rounded or oval, lightly staining but well fixed. Although the cells are in many instances vesicular in appearance, they form a solid clump with no sign of cavity formation.

THE INTRA-EMBRYONIC MESODERM

Except in the region of the median plane caudal to the prochordal plate, the intra-embryonic mesoderm forms a continuous open meshwork over the whole of the embryonic area. Round the periphery of the shield the cells are not very numerous but they are connected to one another by branching processes. Wherever they reach the periphery of the shield they become continuous with the primary mesoderm and at the caudal end they pass into the connecting stalk. In places the mesoderm cells fill up the whole interval between the ectoderm and the entoderm, but as a general rule shrinkage spaces intervene between the mesoderm and one or both of the two other

layers. In many situations the mesoderm cells appear to be springing directly from the deep surface of the shield ectoderm and elsewhere they are often connected to it by fine cytodesmata; in other situations they are intimately related to and apparently continuous with the entoderm of the roof of the yolk-sac. There is no evidence in H.R. 1 to show whether these cells are actually derived from the primitive streak, the primary mesoderm, the shield ectoderm or the entoderm. Stieve (1926) believes that the intra-embryonic mesoderm at this stage is derived (1) from the primary mesoderm, (2) from the primitive streak and (3) from the entoderm of the roof of the yolk sac. The histological appearances, however, may be very misleading and I am of opinion that the Embryo H.R. 1 provides no definite evidence in this connexion.

Typical mesoderm cells can be seen in Text-figs. 23 and 24 apparently arising from the caudal end of Hensen's node, and others are seen in Text-figs. 21 and 22, apparently arising from the cranial end of the node and lying dorsal to the left edge of the head process.

To the right of the median plane the sections become more oblique owing to the curvature of the shield. Two consecutive sections pass between the right wall of the yolk-sac and the shield ectoderm (Text-fig. 30). In these the fusiform cells of the intra-embryonic mesoderm form a continuous open-meshed network from the cranial to the caudal periphery of the embryonic area by means of their interconnected branching processes.

In sections 18·9–19·3 the exocoelom encroaches on the embryonic area at the cranial end of the shield and the primary mesoderm which lines the invagination is directly continuous with the intra-embryonic mesoderm, so that it is impossible to be certain where the one ends and the other begins. In the two succeeding sections the intra-embryonic mesoderm at the cranial end of the shield contains a cavity which appears to be shut off from the exocoelom, which no longer encroaches on the embryonic area. In the nine succeeding sections the same cavity is present (Pl. 1, fig. 2), although it is partly interrupted in 19·8 and is damaged in 19·11, and it extends cranially practically to the limit of the shield. This cavity-containing mesoderm extends caudally to the region of the prochordal plate.

Only two possible interpretations can be suggested for this cavity, for the appearances exclude the possibility of vascular formation. Either it is an artefact due to shrinkage, or it is a precociously developing coelomic space. At first sight the former explanation is the more probable. No trace of any such cavity is present in other embryos at this stage.[1] In the Dobbin embryo (Hill & Florian, 1931), which is 0·96 mm. long, the minute cavities apparent

[1] In the embryo described by Schlagenhaufer & Verocay (1916), which is only 0·24 mm. long, mention is made of a cleft in the intra-embryonic mesoderm near the cranial end of the shield. The cleft appeared in one section only and was not figured. As the description is very incomplete it is impossible, without examining the sections, to come to any conclusions about it. It is regarded by the authors as suggestive of a coelomic formation.

in two of the sections are regarded by the authors as very doubtful coelomic formations. On the other hand the appearances in H.R. 1 are strongly suggestive of coelomic formation. The continuity of the cavity through so many sections alone throws grave doubt on the likelihood that we are here dealing with an artefact. In some of the sections cytodesmata connect the dorsal wall to the shield ectoderm, but no cytodesmata are found within the cavity. In two of the sections (19·12 and 20·1) the walls of the cavity present the "syncytial ribbon" appearance which has already been shown to be indicative of good fixation and preservation. One is therefore forced to conclude that the cavity is a natural space within the intra-embryonic mesenchyme and as such it can only represent a precociously developed coelomic space.

THE PRIMARY MESODERM

On the amnion the primary mesoderm forms a single layer of cells, except around the periphery of the embryonic shield where it forms a rather thicker layer on the upturned rim. Towards the cranial and caudal limits of the amniotic roof no difficulty is experienced in distinguishing the mesodermal from the ectodermal layer. The mesodermal cells are fusiform with prominent nuclei: the ectodermal cells also are elongated but their nuclei do not project above the general level. Over the central part of the roof the two layers are indistinguishable and in the sections they form a ribbon-like syncytium (Text-fig. 23). The same appearance has already been noted in the wall of the coelomic space and in the wall of the yolk-sac. In the latter situation and in the amniotic roof similar areas are present in both the Strahl-Beneke and the Hugo embryos. The condition has been discussed at an earlier stage.

On the yolk-sac two types of cells can be identified without difficulty. The first type, which greatly exceeds the second in number, comprises cells which are almost epithelioid. Their nuclei are rounded and relatively large; they do not stain darkly but their nucleoli are conspicuous and they contain a fine chromatin network. In some places the cells of this type form a single layer, but in many others they form clumps of variable size, containing from five to thirty cells. The second type comprises a number of elongated, flattened cells. They are found in places covering the entoderm but they are more commonly situated amongst the clumps of cells of the first type, where they help to form the walls of intercellular spaces.

These clumps are regarded as blood islands and the occasional spaces as early stages in the formation of vascular spaces. In the islands the cell boundaries are indistinct and the cytoplasm is pinkish in tint. The vascular spaces are neither so large nor so numerous as those found in the same situation in the Embryo Hugo; they are all small in size and they do not communicate with one another.

The connecting stalk extends from the apical part of the chorionic vesicle to the caudal end of the embryonic area and the adjoining part of the roof of the amnion. Attention has already been drawn to the gap which is apparent

between the connecting stalk and the dorsal surface of the funnel-shaped diverticulum from the caudal surface of the yolk-sac. Although, as stated, the gap is regarded as a tear in the embryonic attachment of the connecting stalk, the appearances of its boundaries would lead one to suppose that it is a natural interval, and the evidence in favour of the interpretation put forward is mainly of a negative character. If it is not a tear, then the connecting stalk has a very narrow attachment to the embryo and a very wide attachment to the amnion, a condition which is not impossible but which is exceedingly improbable. The allantoic cord has a pronounced bend and its connexion with the yolk-sac has been displaced considerably to the left of the median plane. Such a condition can be explained satisfactorily by a tear of the connecting stalk, as already indicated. Finally, no corresponding gap is present in other early human embryos.

The ab-amniotic surface of the stalk is covered with loosely arranged and loosely connected epithelioid cells which occasionally form small surface clumps very similar to the blood islands on the yolk-sac. As the stalk is traced towards its chorionic attachment it becomes less cellular and is looser in texture. Actual spaces—which are rare in the vicinity of the embryo apart from contraction spaces—are more numerous and are relatively large, much larger than the spaces in the wall of the yolk-sac. Many of them are surrounded by flattened cells which indicate their vascular character (Text-fig. 34).

As already stated, the connecting stalk contains the allantoic cord and the whole extent of the cloacal membrane.

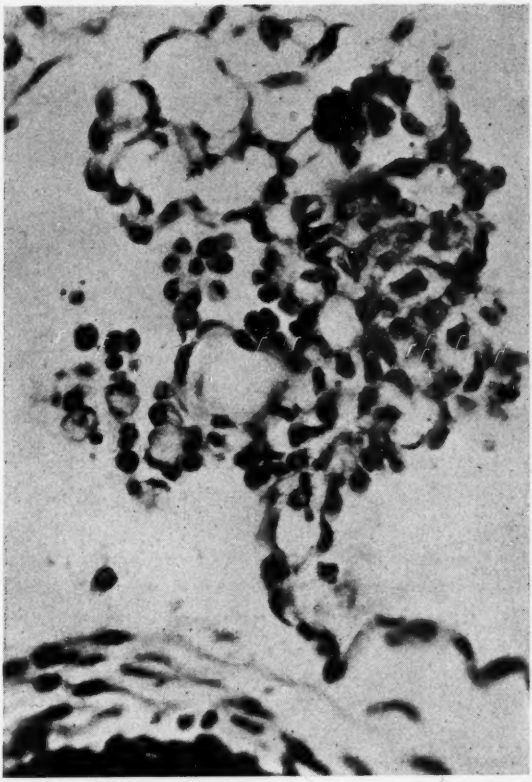
THE AGE OF THE EMBRYO

The temptation to regard the dimensions of the embryonic shield as a reliable guide to the fertilization age of the embryo has disappeared since Stieve (1926) published his description of the Embryo Hugo. In that case a period of only $13\frac{1}{2}$ days elapsed between insemination and operation. If only 12 hr. is allowed for the interval between insemination and fertilization, the fertilization age of the Embryo Hugo would be 13 days. In Bryce's Embryo T.B. 1 (1908) the history was not less reliable than in the Embryo Hugo. An interval of $16\frac{1}{2}$ days elapsed between insemination and the abortion. Bryce allowed 24 hr. for the interval between insemination and fertilization and a period of 24–36 hr. between the death of the ovum and the actual abortion, and finally concluded that the fertilization age of the embryo was 13–14 days. Thus 13 days may be regarded as the minimum for T.B. 1 and as the maximum for the Embryo Hugo. But the developmental stage of T.B. 1 is very considerably earlier than that of the Hugo, and according to Bryce's reckoning the latter would take its place in his series between the Leopold and the Reichert at an estimated age of 17–18 days.

Consideration of these two embryos makes it abundantly clear that the rate of development is by no means constant for human embryos and that two

specimens of the same fertilization age may differ in developmental stage by as much as 3 or 4 days. Such a disparity had been foreshadowed by Rabl (1915), who showed that at 7 days 8 hr. fertilization age, rabbit embryos of the same litter might differ in shield length by as much as 50 %.

In the Embryo H.R. 1 unfortunately no evidence is available as to the actual fertilization age, but the developmental stage is clearly earlier than that



Text-fig. 34. The connecting stalk in section 20·10. ×400.

of the Embryo Hugo and as clearly later than that of the Embryo Strahl-Beneke or the Embryo T.F. The operation of hysterectomy was performed on the day before the next period was expected to begin, or 27 days after the commencement of the last period. As the normal duration of the periods was 6–7 days, the maximum fertilization age would be 19 days, if 24 hours are allowed as the interval between insemination and fertilization. Assuming a developmental rate as rapid as that of the Embryo Hugo, the minimum

fertilization age would be in the neighbourhood of 12½ days. The actual fertilization age lies somewhere between these two extremes and is probably about 15½ days.

Is the Embryo H.R. 1 *a normal embryo?* This question must arise and deserves careful consideration in connexion with every young human embryo, and it is often exceedingly difficult to answer. Evidence is gradually accumulating to show that even the median plane structures are by no means constant in their relative times of appearance. This variability has been stressed by Grosser (1931*b*, *c*) in two recent papers, and must be borne in mind when coming to a decision with regard to the Embryo H.R. 1.

Our knowledge of the early stages of the human embryo is based on material obtained from one of three sources, viz.: (*a*) from operations, (*b*) from abortions, and (*c*) from post-mortem examinations. In groups (*a*) and (*b*) the condition of the endometrium must always be suspect, for, presumably, the specimens would not otherwise have been obtained. However, the condition of the endometrium in group (*a*) can always be examined in the neighbourhood of the ovum, and in H.R. 1 it displayed no obviously pathological features. The specimens falling into group (*c*) may or may not be free from suspicion as regards the condition of the endometrium, but they nearly always give rise to doubts and difficulties because of their imperfect preservation due to the interval between the occurrence of death and the performance of the autopsy.

In H.R. 1 the pronounced curvature of the shield, which has influenced the size of the yolk-sac, must be regarded as the result of an abnormal growth process. It may have been exaggerated during fixation, but probably not to any great extent. Does this abnormality of shape imply that no importance attaches to any of the features which this embryo displays? Almost certainly not, for careful comparison with other embryos has shown that the median plane structures, with the exception of the coelomic cavity, are in a stage of development which are in harmony with the known facts and with one another. The primitive streak and its two nodes, the head process, the prochordal plate and the cloacal membrane can all be regarded as normal, with the possible exception of the head process. The presence of a coelomic cavity must be regarded as evidence of precocity only, for it conforms in every way to the conditions which our knowledge of its normal development would have led us to anticipate.

To sum up, the Embryo H.R. 1 is admittedly abnormal in shape and is precocious so far as coelomic development is concerned, but in other respects, with the possible exception of the head process, it is normal; and the evidence which it supplies in connexion with the development of the cloacal membrane, the prochordal plate and the allantois is entitled to its fair share of weight.

SUMMARY

1. The Embryo H.R. 1 is at approximately the same stage of development as Grosser's H.Schm. 10. It is at a later stage than the Strahl-Beneke and the T.F. embryos, and at an earlier stage than the Embryo Hugo.

2. The curvature of the shield is the result of a growth process, although it may have been slightly exaggerated post-mortem. It is regarded as an indication of abnormal growth due, in part at least, to the shape of the chorionic cavity.

3. The primordium of the head process is present and exhibits an early stage of development. It consists of a group of columnar cells which replace the entodermal roof of the yolk-sac over a limited area.

4. The allantois is represented by a solid entodermal cord, which is regarded as the normal condition of the first stage of this structure.

5. The primordium of the prochordal plate is present.

6. The cloacal membrane is situated beyond the caudal end of the shield. It is suggested that the membrane normally develops first in this situation and then extends cranially to involve the shield.

7. The primitive streak does not extend to the caudal limit of the shield. An early stage in the development of Hensen's node is described.

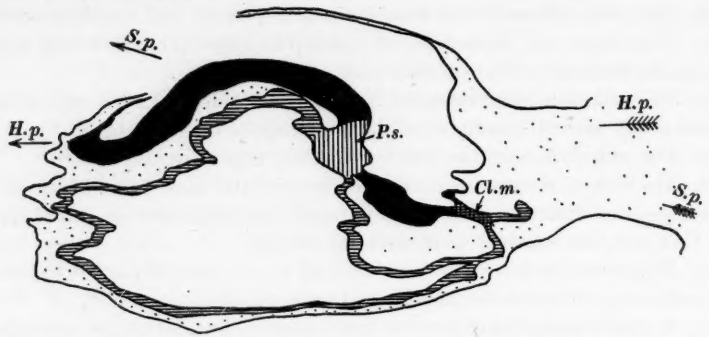
8. A precociously developed coelomic space is present in the cranial part of the shield. The appearances suggest that the space communicates caudally with the exocoelom.

I have to acknowledge gratefully my indebtedness to the University of Edinburgh for granting me permission to publish this communication, which, with slight modifications, was submitted in 1937 as part of a Thesis for the degree of M.D. I am also indebted to Mr G. A. Walker, senior technical assistant in the Anatomy Department, Guy's Hospital Medical School, who was responsible for the micro-photographs, and to Miss P. M. Larivière, who executed the line drawings. I also acknowledge with gratitude the fact that the expenses of illustration have been defrayed in part by a generous grant from the Clinical Research Committee of Guy's Hospital Medical School.

NOTE

The sections were submitted to Prof. Florian, who had seen the photographs and had indicated his interest in the specimen. Early last year he sent me a graphic reconstruction of a median section—made under circumstances of quite extraordinary difficulty. I am very greatly indebted to him for the time and trouble he has devoted to this embryo and for the interest he has taken in it. Unfortunately, for obvious reasons, I have not had an opportunity of discussing with him the points on which our reconstructions differ, and I have therefore not included reference to his reconstruction in the general argument. I feel, however, that it is only fair to readers of this paper to include the figure (Text-fig. 35) which Prof. Florian has given me per-

mission to publish, and the measurements made from it are given in Table 1. It must be emphasized that the curvature of the embryo and the obliquity of the sectional plane make accurate reconstruction difficult by any method, and these difficulties are increased by the partial tear in the connecting stalk and the consequent kinking at the caudal end of the embryo (p. 6). It is not surprising, therefore, that Prof. Florian and I are at variance as to the inclination of the sectional plane, and this accounts for the difference in our estimates of the length of the shield.



Text-fig. 35. Graphic reconstruction of a median section through H.R. 1 made by Prof. J. Florian. ×150. Solid black = undifferentiated ectoderm: horizontal lines = ectoderm. *P.s.* = Primitive streak; *Cl.m.* = Cloacal membrane; *S.p.* = Sectional plane; *H.p.* = Plane of Prof. Florian's dorsal projection (not figured).

Further, what I have identified as Hensen's node, Prof. Florian regards as the primitive streak at an early stage of differentiation, and what I have identified as the primitive streak, he regards as undifferentiated shield ectoderm. Lastly, Prof. Florian does not recognize either a head process or a prochordal plate in H.R. 1. Readers will be able to form their own opinions by examination of the figures.

The other differences between the two reconstructions are of minor importance.

On the other hand, the two reconstructions are in agreement as to the general shape and outline of the shield, the allantoic primordium and the position of the cloacal membrane.

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Fig. 1.

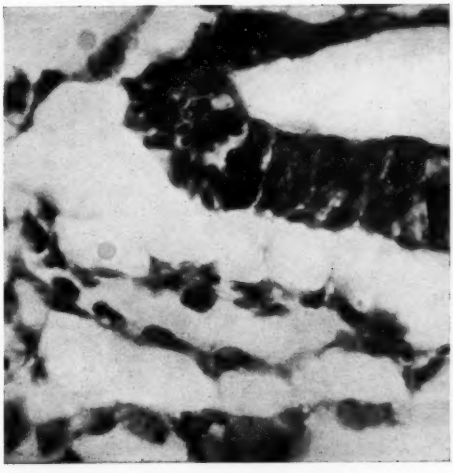

Fig. 2.

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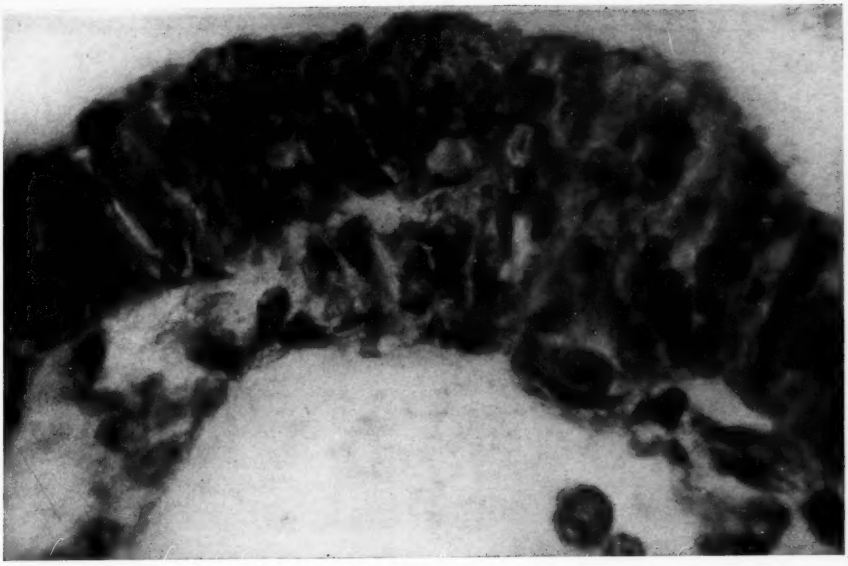

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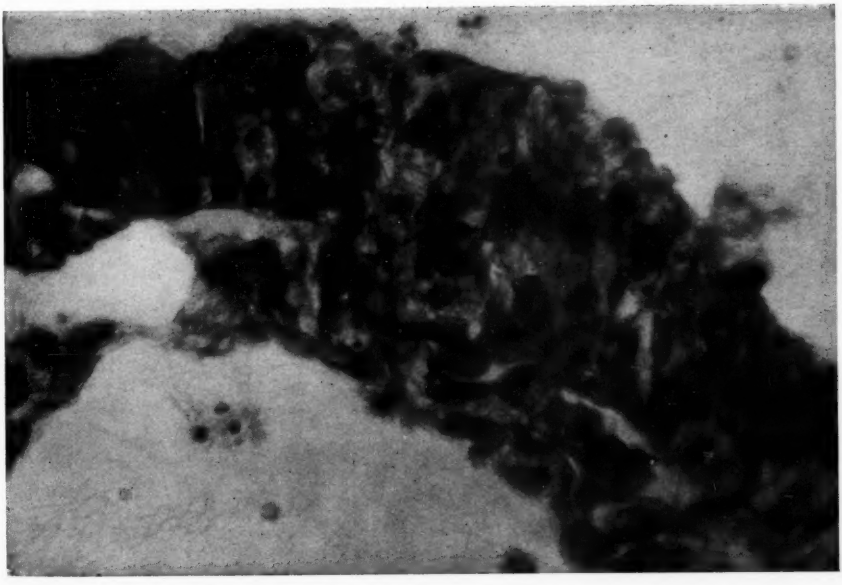

Fig. 2.

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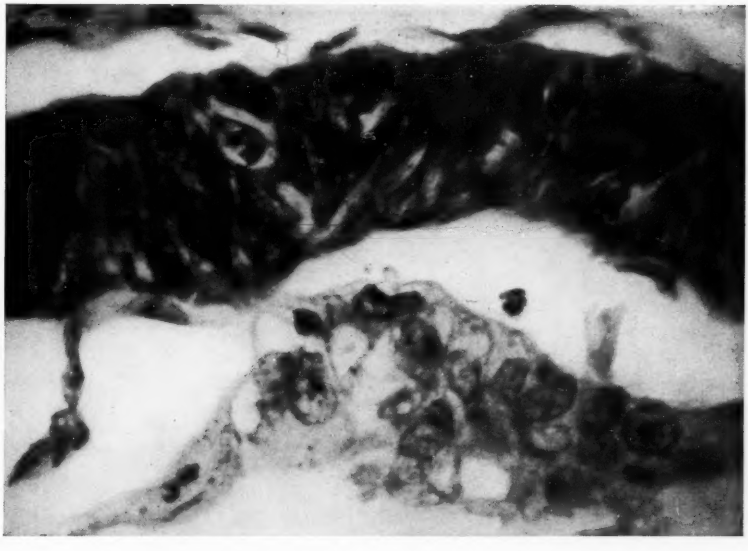

Fig. 2.

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EXPLANATION OF PLATES 1–3

PLATE 1

Fig. 1. The ovum *in situ*, showing the triangular shape of the chorionic cavity, the position of the embryo within the chorion, the villi and intervillous space and the large blood clot which obscures the point of entry. The dilated uterine glands in the stratum spongiosum are conspicuous and attention is drawn to the difference between those in the immediate vicinity of the ovum and those farther distant. ×16.

Fig. 2. Part of section 19·6 showing the cranial end of the shield and the (?) coelomic space. ×634.

PLATE 2

Fig. 1. Part of section 19·11 showing Hensen's node and the head process. (Cranial end of shield to left and caudal end to right.) ×950.

Fig. 2. The corresponding part of section 19·12. ×950.

PLATE 3

Fig. 1. Part of section 19·12 showing the patch of thickened entoderm identified as the prochordal plate. Note its connexion with the shield ectoderm. ×950.

Fig. 2. The corresponding part of section 20·1. ×950.

A MACROSCOPIC STUDY OF THE NERVE SUPPLY OF THE STOMACH

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The stomach is supplied by nerves which arise from various sources and pursue different pathways, and in this respect it resembles all the other important viscera, for it is becoming increasingly evident that multiple innervation is a characteristic feature of the autonomic system. The following observations are based on a series of fifteen dissections of infants, performed with the aid of a dissecting microscope, and it will be convenient to describe the sympathetic and parasympathetic supplies separately.

SYMPATHETIC

With certain exceptions, the great majority of the sympathetic filaments which supply the stomach are derived from the coeliac plexus. The exceptions are as follows:

(1) Filaments from the thoracic ganglionated trunks or splanchnic nerves supply the lower end of the oesophagus, and some of these may reach the cardia by passing down alongside the oesophagus (Text-fig. 1). Their insignificant size in most cases, however, is evidence of their relative unimportance.

(2) Other sympathetic fibres may be contributed by the infra-diaphragmatic part of the left greater splanchnic nerve, or by the upper end of the left lumbar ganglionated trunk. These usually unite with the gastric branches of the left phrenic plexus, or they may pass directly to the cardiac orifice.

(3) I have suspected for some years that the left phrenic nerve contributed branches directly to the stomach, probably sympathetic in nature, but only recently has it been possible to demonstrate the supply with absolute certainty. In this specimen (Text-fig. 2) the phrenic nerve pierced the diaphragm in the usual manner and broke up into three main branches, one passing to the right, one to the left, and one downwards and backwards. The last-named branch accompanied the left phrenic artery and soon gave off a filament on the left side to the diaphragm. A short distance below this point it bifurcated unequally, the larger filament entering the fibres of the right crus near the oesophageal hiatus, and the smaller filament continuing on its course alongside the vessel. About the level of the upper pole of the left suprarenal gland the latter nerve divided into two parts, one of which passed directly to the stomach, entering the viscus near the cardiac orifice. The other twig supplied the left suprarenal gland, and a very fine filament accompanied the artery to the coeliac plexus.

The gastric branches from the coeliac plexus accompany the vessels supplying the stomach, and the maximum number are found alongside the left gastric, hepatic, and phrenic arteries, but others accompany the splenic, right gastric, and gastro-epiploic vessels. Although the actual arrangement is fairly constant, in some cases the left gastric branches are most prominent, and in others the hepatogastric or some of the other gastric nerves, so that the relative importance of the supplies apparently varies in different specimens. Considering that they all arise from the same peripheral source and that a number of alternative pathways exist, this variation is not surprising.

(1) *The left gastric plexus*

The left gastric branches, one to four in number, lie near the artery, and as they approach the cardiac orifice they divide into a small proximal group for the cardiac end of the stomach, and one or two larger branches which run distally, between the layers of the lesser omentum, along the lesser curvature of the viscus (Text-figs. 3, 4; Pl. 1). The terminal twigs accompanying the main branch of the left gastric artery did not reach the pylorus in any specimen, but united with the nerve filaments lying alongside the right gastric artery. One of the proximal branches often encircles the cardiac orifice anteriorly to unite with a branch derived from the left phrenic plexus.

(2) *The hepatic plexus*

The hepatic plexus invariably supplies branches to the stomach, and they reach the organ along several channels:

(*a*) *The hepatogastric nerves.* The main supply consists of two or more filaments which arise from the portion of the hepatic plexus lying in the right margin of the lesser omentum, and pass to the lesser curvature between the layers of the lesser omentum, running parallel to, and often communicating with, the hepatic branches of the anterior vagal trunk which are running in the opposite direction (Text-figs. 1, 3; Pls. 1, 2). Reaching the lesser curvature they may supply filaments directly to the stomach, but they mainly unite with and reinforce the branches of the left gastric plexus. The uppermost branch supplies the gastro-oesophageal junction, and sometimes forms a loop in front of the cardiac orifice by uniting with a branch from the left phrenic plexus.

I suggest the name *hepatogastric* for these nerves, because they are gastric branches of the hepatic plexus, and they reach the stomach by passing between the layers of the hepatogastric ligament.

(*b*) *The right gastric nerves.* Other filaments from the hepatic plexus run with the right gastric artery, and they are more delicate than their left counterparts (Text-figs. 3, 5; Pls. 1, 2). They supply the upper parts of the pyloric region, and some filaments anastomose with the other gastric nerves nearby and with the terminal branches of the left gastric plexus.

(*c*) *The right gastro-epiploic nerves* come from the hepatic plexus via the gastroduodenal artery (Text-figs. 5, 6; Pl. 2). They are larger than the left gastro-epiploic branches, and there may be a single larger nerve or several smaller filaments. They accompany the vessels closely, and send branches upwards to the stomach, and filaments downwards alongside the epiploic vessels to the transverse colon.

(3) *The left phrenic plexus*

The left phrenic plexus supplies several filaments to the cardiac end of the stomach, and one of these is sometimes larger than the others, and passes around the left and anterior aspects of the cardiac orifice to unite with a filament from the left gastric plexus, or the uppermost gastric branch from the hepatic plexus (Text-figs. 1, 3; Pl. 1).

(4) *The right phrenic plexus*

The right phrenic plexus may supply the stomach and liver in the following curious manner, an arrangement which has been seen so far in four cases. One set of delicate nerves accompanies the right phrenic artery closely, but there may be another larger nerve arising from the upper part of the right coeliac ganglion, which lies a little apart from the artery on its inner side (Text-fig. 4). This latter nerve ascends towards the caval foramen, turns to the left behind it, and passes towards the oesophageal hiatus, being closely applied throughout this part of its course to the diaphragm. It supplies one or more slender twigs to the cardiac orifice, but the greater part of the nerve turns forward and passes between the layers of the upper part of the lesser omentum to enter the porta hepatis. In the region of the caval foramen it communicates with a branch or branches of the right phrenic nerve, and the phrenic ganglion is often found on, or close to, these anastomotic branches (Text-fig. 4).

(5) *The splenic plexus*

Delicate terminal branches of the splenic plexus accompany the short gastric and left gastro-epiploic arteries to the stomach, but in two cases a larger single filament was discovered arising above the superior border of the body of the pancreas (Text-fig. 7). This nerve passed upwards behind the lesser sac, to the left side of the left gastro-pancreatic fold, and then turned forwards between the layers of the gastro-phrenic ligament to supply the fundus of the stomach; one of its terminal branches communicated with a gastric branch from the posterior vagal trunk. Swan (1834) noted that the posterior vagal trunk might supply a twig to the splenic plexus, but in my two cases the filament from the splenic plexus was distinctly larger than the communication between it and the gastric branch of the posterior vagal trunk. Therefore part at least of this gastric branch cannot be derived from the vagal trunk, and must contain afferent or efferent fibres passing to or from the coeliac plexus.

PARASYMPATHETIC

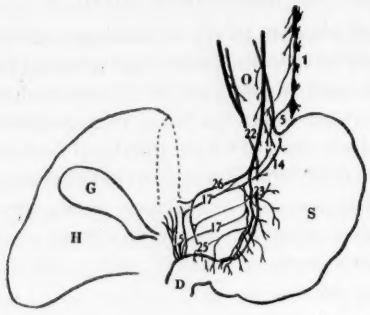
The parasympathetic supply to the stomach is conveyed by the vagi, and it is therefore necessary to describe briefly the terminal parts of these nerves. Considering their size and importance it is curious how many conflicting statements are still current about the lower vagi, and it is a sad reflexion on the neglect of the excellent work of Swan (1834) and Kollman (1860). Perman's (1916), Wertheimer's (1922) and McCrea's (1924) articles are also important contributions, providing not only an analysis of the previous literature, but also records of personal observations. With certain exceptions it has been possible to confirm these workers' findings, and to discover a few more facts about these interesting nerves.

The oesophageal plexus

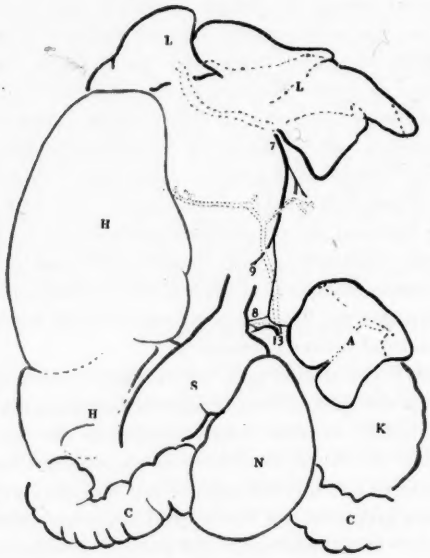
Below the roots of the lungs both vagi usually divide into from two to four main branches which lie in close relationship to the lower oesophagus (Text-figs. 6, 7. 8; Pl. 2). In this series of fifteen dissections the average number of branches on the right side has been three, and on the left side two. The right and left groups of fibres pursue an oblique course, so that the former come to lie on the posterior and the latter on the anterior aspect of the viscus, but one or more branches from the right vagus join the anterior half, and the left vagus contributes one or more branches to the posterior half of the plexus. All these branches are interconnected, so that a simple plexus gulae is formed, with rather open meshes. It is joined by sympathetic filaments from the thoracic ganglionated trunks and greater splanchnic nerves, and gives off numerous filaments which sink into the oesophageal walls. The relative absence of connective tissue between the constituent parts of the oesophageal plexus is rather characteristic, contrasting with the plentiful connective tissue which exists between the component parts of several other autonomic plexuses, such as the coeliac and hypogastric. Thus in most cases the plexus shows up clearly as soon as the mediastinal pleura is removed.

Immediately above the diaphragm, or actually within the oesophageal hiatus, the branches of the plexus reunite into two or more main trunks which enter the abdomen and lie anterior and posterior to the gastro-oesophageal junctions (Text-figs. 7, 8; Pls. 1, 2). There is no constant number, but it is unusual to find more than two on each aspect, and sometimes there is only one. Each trunk contains a proportion of fibres from both vagi, although right and left fibres preponderate in the posterior and anterior trunks respectively.

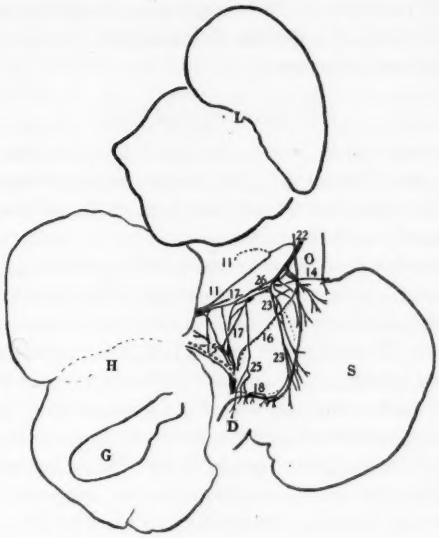
Although the arrangement above described is the commonest, two important variations must be mentioned. The left vagus sometimes passes mainly to the posterior aspect of the oesophagus, and the right to the anterior surface, where they form anterior and posterior plexuses in the usual manner. This has been seen once in thirty-four dissections (the gastric nerves have only been dissected out completely in fifteen cases, but actually thirty-four special



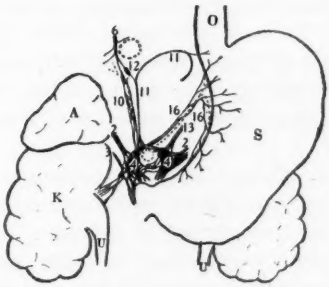
Text-fig. 1. A drawing from the photograph of a dissection. The left lobe of the liver has been removed to show the nerves between the layers of the lesser omentum.



Text-fig. 2. A line drawing from the photograph of a dissection. The left lobe of the liver has been displaced upwards and to the right, and the spleen has been drawn downwards and inwards, while most of the diaphragm has been removed; the two dotted lines to the right of the number 7 represent the cut edge of the diaphragm. The course of the left phrenic artery is indicated by the red dotted lines.



Text-fig. 3. A line drawing of the dissection shown in Pl. 1. The hepatic and right and left gastric arteries are represented by red dotted lines.



Text-fig. 4. A line drawing from the photograph of a dissection. The coeliac artery is represented by a red dotted circle, and the inferior vena cava by a blue dotted oval. The right phrenic and left gastric vessels are indicated by red dotted lines.

dissections have so far been performed). In another specimen no plexus gulae was formed, the two vagi being reconstituted into single trunks below the lung roots, whence they passed on to the oesophagus, the left being anterior and the right posterior (Text-fig. 1). Within the abdomen the two nerves were distributed in the ordinary manner.

The anterior vagal trunk

The anterior vagal trunk, which may be single (ten cases) or double (five cases), supplies several filaments to the cardiac orifice before dividing near the proximal end of the lesser curvature, into four groups of branches: (*a*) gastric, (*b*) pyloric, (*c*) hepatic, and (*d*) coeliac.

(*a*) *Gastric branches* (four to ten) are more numerous than the others and radiate to the lesser curvature, the uppermost fibres supplying the anterior aspects of the cardiac orifice and fundus, and the lowermost fibres reaching almost to the pylorus (Text-figs. 1, 5, 8; Pls. 1, 2). Frequently one branch is larger than the others and lies along the anterior part of the lesser curvature, between the layers of the lesser omentum, where it communicates by delicate tendrils with the adjacent sympathetic nerves and with the corresponding branch of the posterior vagal trunk (Text-figs. 1, 5, 8; Pl. 1). Latarjet (1921) and his co-workers describe this nerve as *the principal anterior nerve of the lesser curvature*, a clumsy appellation, but one which indicates its usual predominance in size; I suggest the term *greater anterior gastric nerve*, although this is also somewhat clumsy. The various gastric branches can be traced for some distance beneath the serous coat before they sink into the stomach musculature, and, although they communicate with neighbouring gastric nerves, I have never seen a true anterior gastric plexus either in these special dissections or in ordinary dissecting-room subjects. This is a point about which there is wide divergence of opinion, Swan (1834), Kollman (1860) and Spalteholz (7th English edition, no date) amongst others stating that plexus formation does occur, while Sappey (1872), Schäfer & Symington (1909), and Perman (1916) say there are no true gastric plexuses. McCrea (1924) found anterior plexuses in certain cases, but he remarks that the opinion of the individual observer as to what constitutes a plexus apparently varies, and thus the discrepancies may be explained.

(*b*) *Pyloric branches.* Pyloric branches are invariably supplied by the anterior vagal trunk, two being the common number (Text-figs. 1, 8; Pls. 1, 2). One arises high up near the left end of the lesser curvature, passes almost horizontally across between the layers of the lesser omentum towards its free edge, and then turns downwards on the left side of the hepatic artery to reach the pylorus and proximal part of the duodenum. It often bifurcates, sending one branch upwards to the liver and the other downwards to the pylorus, and it is always connected to the hepatic plexus by very fine fibres. The other pyloric branch usually arises from the greater anterior gastric nerve, about half-way along the lesser curvature, and it passes obliquely between the layers

of the lesser omentum to the pyloric antrum, some fibres reaching the pyloric sphincter. This branch thus arises at a lower level, but supplies the pylorus proximal to the branch first described.

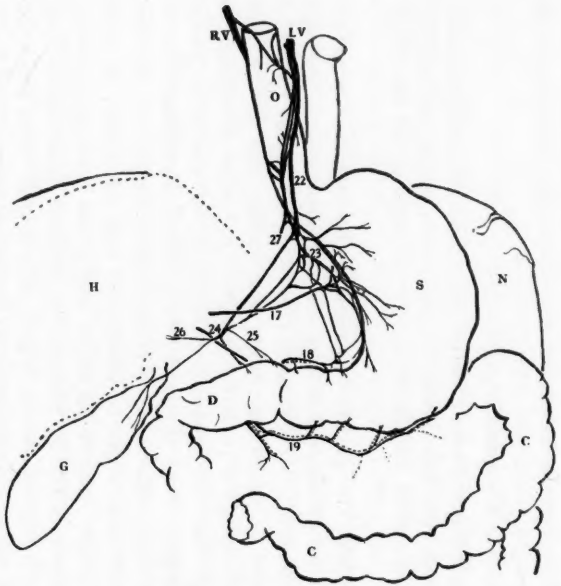
(*c*) *Hepatic branches.* Hepatic branches (two to four) are given off both from the stem of the anterior vagal trunk and from the greater anterior gastric nerve (Text-fig. 1; Pls. 1, 2). They pass to the right, and often blend with branches of the hepatic sympathetic plexus, but in some cases they pass directly to the porta hepatis without any significant communications with this plexus; the upper hepatic twig in particular often pursues an independent course. The nerves frequently divide on the left side of the hepatic artery, one branch passing upwards to the liver, and the other downwards to the pylorus or the coeliac plexus, while in one specimen the uppermost hepatic branch divided into three twigs; the first entered the porta hepatis, the second passed on to the cystic duct and gall-bladder, and the third descended alongside the hepatic artery to the pylorus (Text-fig. 5; Pl. 2).

(*d*) *Coeliac branches.* The anterior vagal trunk always sends twigs to the coeliac plexus, but judging from their size they only account for about one-quarter or one-third of the anterior trunk fibres, whereas the coeliac contribution from the posterior trunk usually represents about two-thirds of its bulk. The main coeliac branch(es) of the anterior vagal trunk arises near the cardiac orifice (Text-fig. 5; Pl. 2) and passes downwards with the left gastric artery, frequently blending with the corresponding coeliac division of the posterior vagal trunk. Other contributions to the coeliac plexus come from the hepatic or pyloric branches of the anterior trunk, and they reach the plexus alongside the hepatic artery.

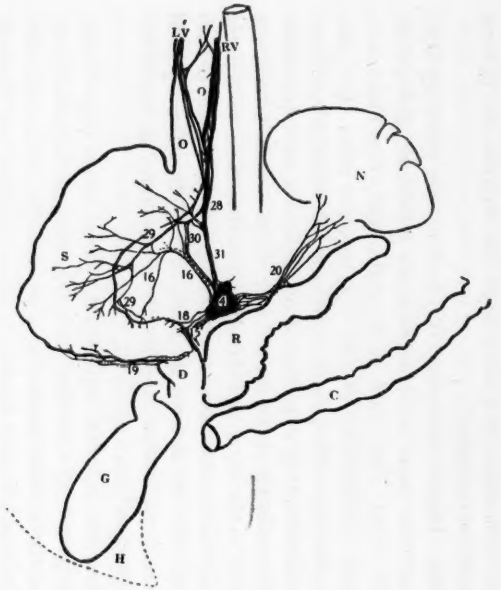
The posterior vagal trunk

The posterior vagal trunk(s) may be single (eight cases), double (six cases), or triple (one case). Two main sets of branches, gastric and coeliac, are supplied by this trunk, and the coeliac division is usually the larger of the two (Text-figs. 6, 7).

The gastric branches. The gastric branches of the posterior trunk are distributed in a manner reminiscent of their anterior counterparts, although their area of supply is slightly less extensive (Text-figs. 6, 7). Thus a group of fine filaments radiates to the cardiac orifice and fundus, and one or more larger nerves proceed along the posterior margin of the lesser curvature. As on the anterior aspect there are usually several nerves, but one of the groups is always larger than the others. Latarjet (1921) and his co-workers refer to this nerve as *the principal posterior nerve of the lesser curvature*, but the term *greater posterior gastric nerve* might be substituted with advantage. In some dissections it forms a very conspicuous object, and in one specimen it seemed to account for about one-half of the fibres of the posterior vagal trunk (Text-fig. 6); not far from its origin, however, it gave off a branch which proceeded downwards alongside the left gastric artery to the coeliac plexus (Text-fig. 6). This arrangement has been seen in several other specimens—namely that a



Text-fig. 5. A line drawing of the dissection shown in Pl. 2. The right gastric and gastro-epiploic vessels are indicated by red dotted lines.



Text-fig. 6. A line drawing from the photograph of a dissection. The stomach has been turned over to the right so that the posterior surface is exposed. The left half of the liver has been removed and the remainder pulled downwards. The gastric and right gastro-epiploic arteries are indicated by red dotted lines. To prevent confusion, gastric branches of the anterior vagal trunk and of the left gastric and hepato-gastric nerves have not been filled in.

Text-fig. 7. A line drawing from the photograph of a dissection. The stomach has been partly rotated to the right in order to show the posterior surface. The left lung has also been displaced to the right to show the cardiac, pulmonary, aortic and oesophageal nerves.

Text-fig. 8. A line drawing from the photograph of a dissection. The left lung has been turned to the right and the oesophagus has been displaced slightly to the left to display the posterior half of the oesophageal plexus. Note the plexus form assumed by the coeliac division of the posterior vagal trunk; careful dissection reveals that this is a common occurrence. To minimize confusion the left gastric nerves are not indicated.

bundle of fibres, ultimately destined for the coeliac plexus, runs for a short distance as a constituent part of the greater posterior gastric nerve, and then becomes separated from this nerve near the upper end of the lesser curvature. This coeliac contribution sometimes rejoins the main coeliac division of the posterior vagal trunk, or may pursue an independent course, as in the case illustrated. Slender hepatic twigs also arise occasionally from the gastric division, and pass to the porta between the layers of the lesser omentum. The posterior gastric nerve gives off branches at intervals which pass to the stomach, but the terminal branches extend only to the pyloric antrum, failing to reach the pyloric sphincter. They communicate with the adjacent gastric nerves, but in no case was there a true posterior gastric plexus in this series of dissections. In this respect, as in the case of the anterior gastric plexus, there is wide divergence of opinion, some saying there is plexus formation and others that there is none. As these discrepancies are possibly due to the fact that no two observers agree exactly about the size or number of the intercommunications necessary to constitute plexus formation, it should be stated that in this investigation unless a definite nerve network was found, and not merely occasional delicate intercommunications, it was decided that no plexus existed. The gastric nerves usually pursue a relatively independent course, communicating only by filaments distinctly finer than the parent stems, and if this be regarded as plexus formation, then it would be true to say that both anterior and posterior gastric plexuses exist.

The coeliac branches. The coeliac branches are larger than the gastric in most cases, and in only one specimen were the gastric and coeliac divisions about equal in size (Text-fig. 6); in this case, however, as has been already explained, the gastric branch gave off a coeliac contribution near the proximal end of the lesser curvature, and it was stated that similar, though smaller, coeliac branches of the greater posterior gastric nerve have been detected in several other specimens. At first sight the coeliac division appears to consist of a single nerve, but careful dissection shows that it is often composed of three or four branches lying close together, and united by large intercommunications so that a plexus is formed (Text-figs. 6, 8). All branches end in the coeliac plexus, part passing to the right half and part to the left, but so far it has not been possible to determine the ultimate destination of the individual branches because of the multiple intricate interconnexions in the coeliac plexus.

From this account, it will be observed that the variety of sources from which gastric nerves are derived is remarkable, and their number is evidence of their importance.

SUMMARY

1. The stomach, in common with other important viscera, is supplied by nerves from multiple sources.

2. The sympathetic supply is derived mainly from the coeliac plexus, and the

majority of the fibres accompany the various gastric arteries. Other inconstant filaments are derived from the left phrenic and splanchnic nerves and the thoracic and lumbar portions of the left ganglionated sympathetic trunk.

3. Judging by their size, the more important contributions are derived from the left gastric, hepatic and left phrenic plexuses, and the manner in which they reach the stomach is exactly described.

4. The branch of the left phrenic nerve supplying the right crus of the diaphragm has been shown to contribute a direct branch to the cardiac end of the stomach.

5. It is suggested that the important group of branches passing from the hepatic plexus to the stomach between the layers of the hepatogastric ligament should be termed the *hepatogastric* nerves.

6. A special derivative of the right phrenic plexus which pursues a curious course and supplies the cardiac orifice of the stomach and the liver is described and illustrated.

7. The splenic plexus has been found occasionally to contribute another gastric branch, in addition to the short gastric and left gastro-epiploic nerves.

8. The parasympathetic supply is vagal in origin, so the distribution of these nerves in their lower course is discussed.

9. It is pointed out that the normal arrangement of the right vagus passing posterior and the left vagus anterior to the oesophagus may be reversed, and that plexus gulae formation is not invariable, the nerves sometimes becoming reconstituted below the lung roots to form single trunks.

10. It is suggested that the chief gastric branches of the anterior and posterior vagal trunks should be named the *greater anterior* and *posterior gastric nerves*.

11. The distribution of the pyloric branches from the anterior vagal trunk is described, and one case is recorded where a nerve derived from the anterior vagal trunk gave off twigs simultaneously to the pylorus, liver and gall-bladder.

12. No true anterior or posterior gastric plexus has been seen in any dissection.

13. The coeliac division of the posterior vagal trunk is more often a plexus than a single nerve.

14. In some specimens fibres ultimately destined for the coeliac plexus run for part of their course with the greater posterior gastric nerve.

The observations described above have all been checked on the actual dissections by Profs. A. Low, J. R. Learmonth or R. D. Lockhart, and I wish to express my thanks for their valued help. The Medical Research Council have generously defrayed most of the expenses of the investigation. I wish also to acknowledge a substantial grant from the University of Aberdeen towards the cost of the illustrations.

KEY TO ILLUSTRATIONS

1. Ganglionated sympathetic trunk
2. Greater (thoracic) splanchnic nerve
3. Lesser (thoracic) splanchnic nerve
4. Coeliac plexus
5. Filaments of supra-diaphragmatic origin supplying the gastro-oesophageal junction
6. Right phrenic nerve
7. Left phrenic nerve
8. Gastric branch of left phrenic nerve
9. Right crural branch of left phrenic nerve
10. Right phrenic plexus
11. Gastric and hepatic branch of right phrenic plexus
12. Phrenic ganglion
13. Left phrenic plexus (lower part)
14. Loop in front of cardiac orifice connecting gastric branch of left phrenic plexus and uppermost hepatogastric nerve (or a branch of the left gastric plexus)
15. Hepatic plexus
16. Left gastric nerve(s) (plexus)
17. Hepatogastric nerve(s)
18. Right gastric nerve(s) (plexus)
19. Right gastro-epiploic nerve(s)
20. Splenic nerves (plexus)
21. Gastric branch of splenic plexus arising above superior border of body of pancreas
22. Anterior vagal trunk
23. Greater anterior gastric nerve
24. Common nerve supplying liver, gall bladder and pylorus
25. Pyloric branch(es) of anterior vagal trunk
26. Hepatic branch(es) of anterior vagal trunk
27. Coeliac branch of anterior vagal trunk
28. Posterior vagal trunk
29. Greater posterior gastric nerve
30. Coeliac branches arising from greater posterior gastric nerve
31. Coeliac division(s) of posterior vagal trunk

A Suprarenal gland
C Colon
D Duodenum
G Gall bladder
H Liver
K Kidney
L Lung
N Spleen
O Oesophagus
P Pericardium
R Pancreas
S Stomach
T Thyroid gland
U Ureter
RV Right vagus nerve
LV Left vagus nerve

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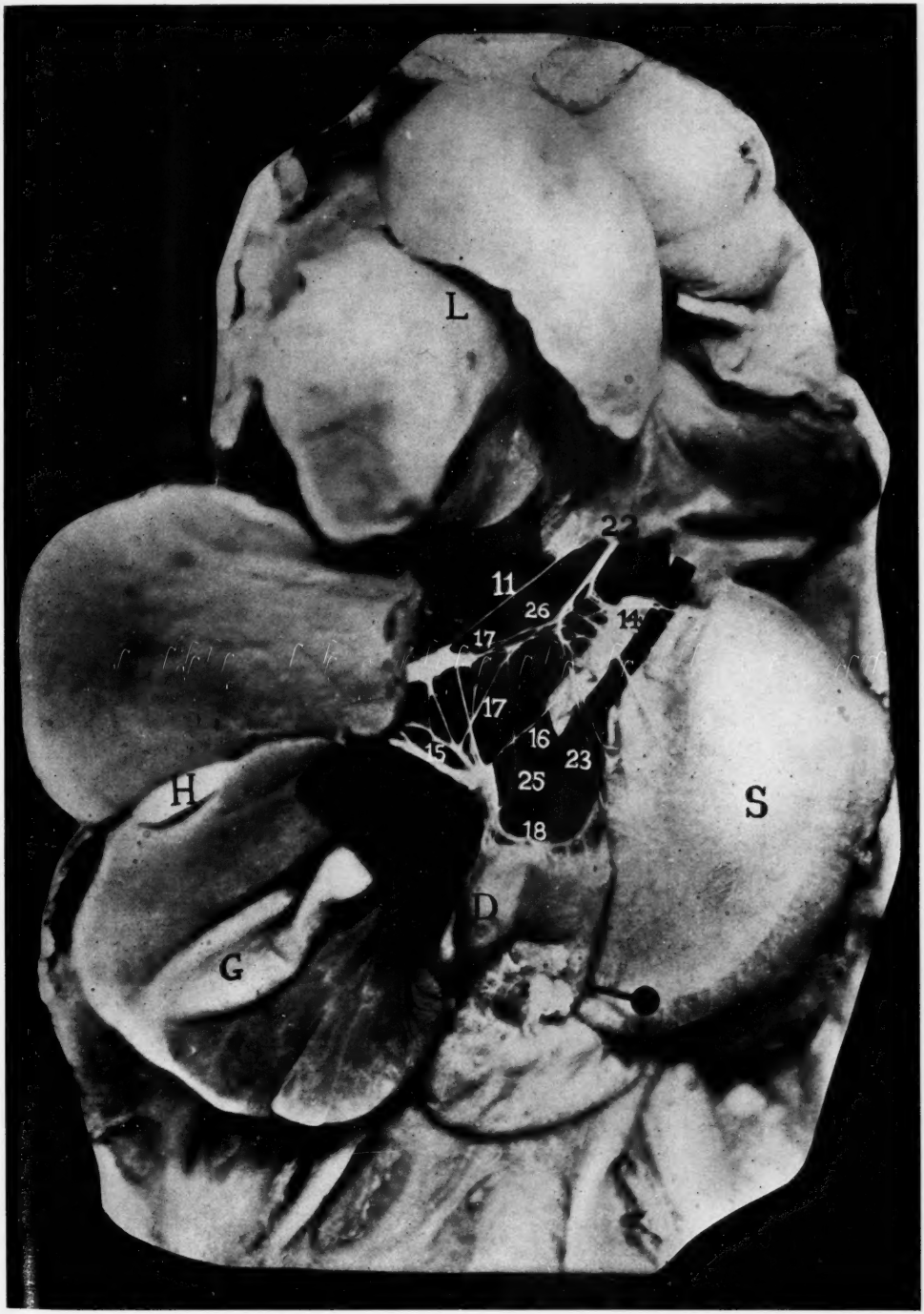

MITCHELL—THE NERVE SUPPLY OF THE STOMACH

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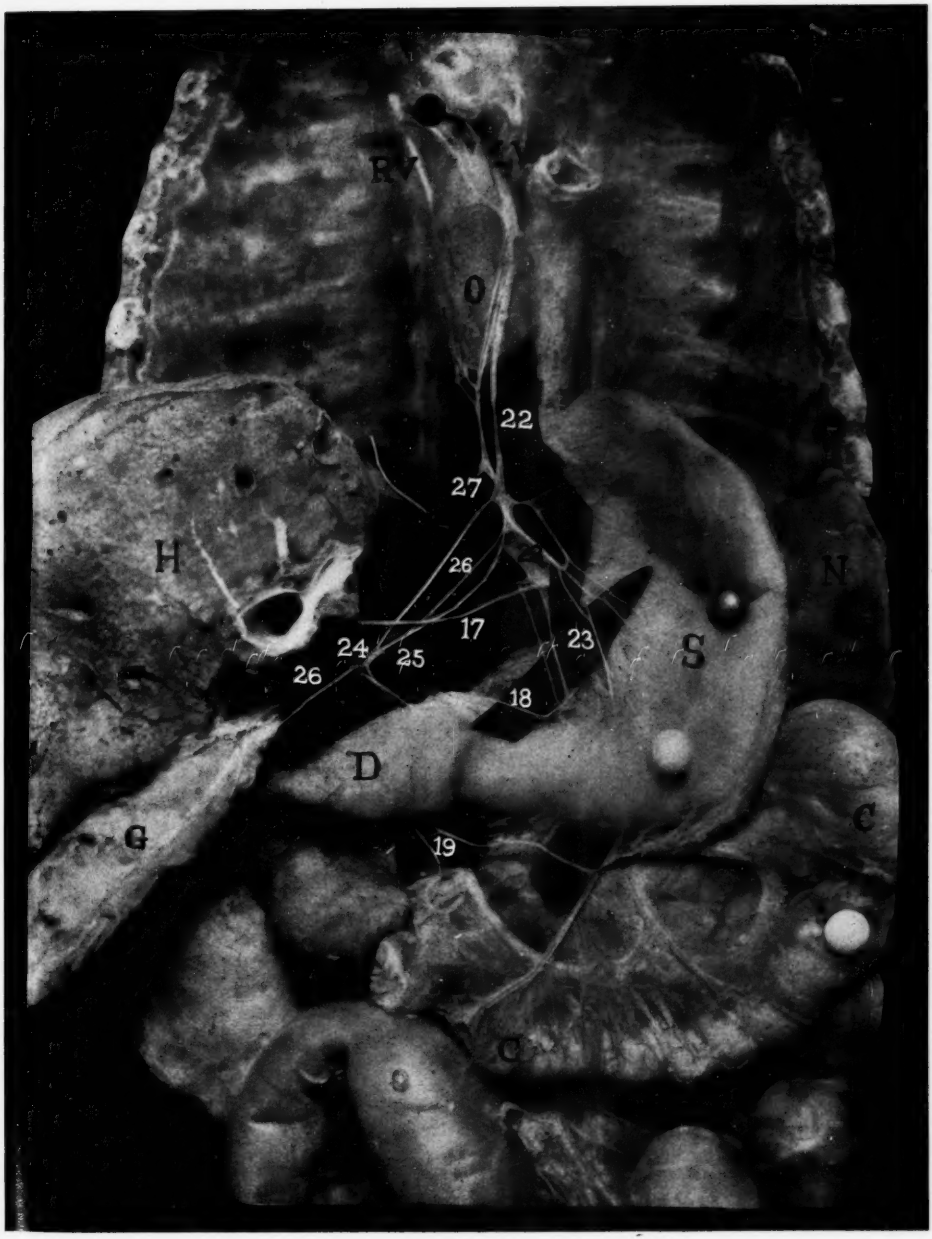

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EXPLANATION OF PLATES 1 AND 2

PLATE 1

In order to expose the cardiac end of the stomach the liver has been rotated slightly, so that its left lobe lies above the right.

PLATE 2

The left half of the liver has been completely removed to display the cardiac end of the stomach and the nerves between the layers of the lesser omentum.

Note. Owing to the high cost of reproduction, it has been possible to reproduce only two of the photographs from which the line drawings were made.

THE NORMAL OCCURRENCE, STRUCTURE AND HOMOLOGY OF PROSTATE GLANDS IN ADULT FEMALE *MASTOMYS ERYTHROLEUCUS* TEMM.

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From the Department of Zoology, University College of North Wales, Bangor, and the Sir Alfred Lewis Jones Research Laboratory, Freetown, Sierra Leone

I. INTRODUCTION

A large and well-developed prostate gland is present in all normal females of the multimammate mouse, *Mastomys erythroleucus* Temm., whether adult or immature. The histological characters of the gland suggest that it is actively secretory and, presumably, functional, though its physiological significance is obscure. It differs both in its size and the uniformity of its occurrence from the rudimentary prostate of some female rats and mice.

The presence of this organ was discovered when the reproduction of *Mastomys* was being investigated in material collected by one of us in the vicinity of Freetown, Sierra Leone (Brambell & Davis, in press). The material available consisted of 104 females that had been trapped, including both immature and adult animals. Some of the material had been sectioned before regular weighing of the whole prostate was instituted. Weights are, therefore, only available for thirty-seven animals. The technique employed has been described elsewhere (Brambell & Davis, in press).

A description of the anatomical and histological characters of the prostate of the male is included as a basis for consideration of the homology of the organ of the female.

II. OCCURRENCE IN OTHER MAMMALS

Small periurethral glands occur in female mammals belonging to several orders and have been recognized as the homologues of part or the whole of the prostate of the male. The earlier literature dealing with the subject has been reviewed by Korenchevsky & Dennison (1936), Lehmann (1938) and Price (1939).

The periurethral glands in women are small, consisting of few or many acini opening into the urethra. They may surround the urethra or may be confined to the side next to the vagina. They are often limited in extent to an area near the neck of the bladder. Histologically they resemble the prostate of the male, with which it has been suggested they are homologous.

Rudimentary prostate glands have been described in the female mole. Accessory glands in the vaginal wall are present in the hedgehog; according

to Deanesly (1934) there are upper and mid-vaginal glands opening into the vaginal lumen. The upper glands are small and situated at the level of the base of the bladder. They are branched, tubular glands lying under the vaginal epithelium. The mid-vaginal glands are larger and extend dorso-laterally on either side of the urethra and a little behind its opening into the vaginal lumen. They consist of masses of closely packed tubules. Both these glands regress in anoestrus. The upper vaginal glands regress, but there is evidence that the mid-vaginal glands become active during pregnancy. The mature female *Hemicentetes* has a pair of large paravaginal glands without ducts (Lehmann, 1938). They are situated mainly outside the muscular sheath of the urino-genital ducts but also extend within it, though they do not open into either the vagina or the urethra. The glands appear to be actively functional and the alveoli are filled with secretion. Histologically and anatomically they resemble the prostate of the male.

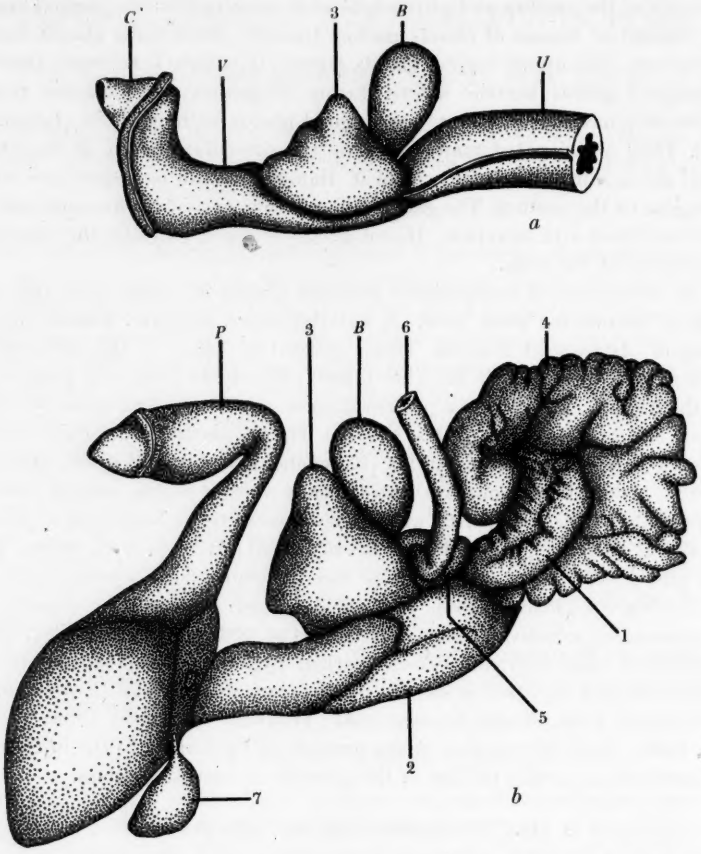
The occurrence of rudimentary prostate glands in female rats and mice has been known for some time. A well-developed prostate, present in the female of *Arvicanthis cinereus* Thos., wedged in front of the neck of the bladder, was described by Rauther (1909). The ducts from this gland open into the urethra. A prostate is present in some female rats and opens by a duct on each side into the urethra (Marx, 1932). The proportion of animals in which it occurs varies in different strains (Korenchevsky, 1937; Witschi, Mahoney & Riley, 1938). The glands are histologically similar to the ventral lobes of the prostate of the male, with which they appear to be homologous (Korenchevsky & Dennison, 1936; Korenchevsky, 1937; Witschi *et al.* 1938). They grow and develop until the age of 40 days in young normal female rats and then retrogress (Price, 1939). The incidence of prostates in females can be increased by selective breeding (Witschi *et al.* 1938; Witschi & Riley, 1939). Injection of male hormone causes marked hypertrophy of the prostate in female rats, but injection of oestrin produces no enlargement (Korenchevsky & Dennison, 1936; Korenchevsky, 1937; Deanesly & Parkes, 1938; Witschi *et al.* 1938). Thus the reaction of the prostate of the female to the injection of sex hormones is similar to that of the prostate in castrated males.

III. THE PROSTATE OF THE MALE

The glands associated with the reproductive tract of the male *Mastomys* resemble those of the mouse (Rauther, 1903; Deanesly & Parkes, 1933) and the rat (Witschi *et al.* 1938), but it is necessary to describe them briefly here for comparison with those of the female.

The ampullary glands (Text-figs. 1*b*, 2) surround the base of each vas deferens, into which the numerous small ducts from their alveoli open from all sides. This region of the vas has an enlarged lumen, into which the opening of the more anterior non-glandular and narrow region projects slightly (Text-fig. 2*e*). The vas deferens opens into the urethra shortly after receiving the ampullary ducts (Text-fig. 3*b*). The ampullary alveoli (Pl. 1, fig. 1) are

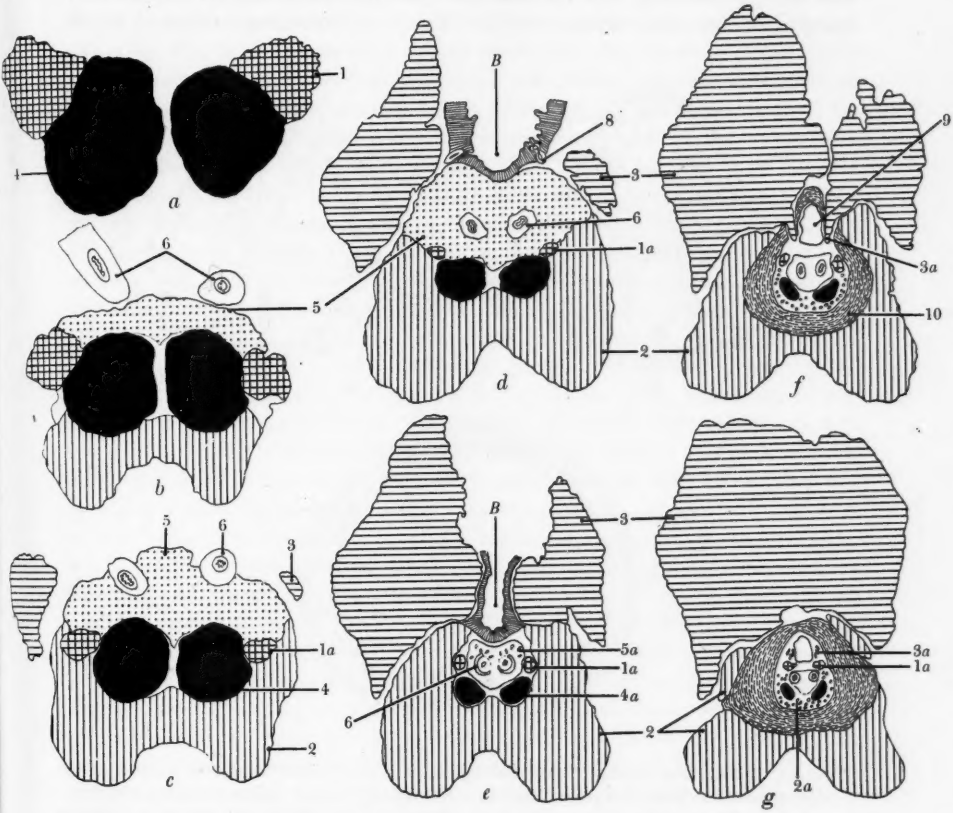
almost circular in cross-section with a simple lumen uninterrupted by any folding of the walls. They are filled with a very dense colloid, intensely eosinophil, which permits of their being distinguished readily in sections from



Text-fig. 1. Drawing of dissections of the reproductive organs of the female (*a*) and the male (*b*) *Mastomys* from the left side. × 2·75. Key to guide letters: 1 = anterior lobe of prostate; 2 = dorsal lobe of prostate; 3 = ventral lobe of prostate of male and prostate of female; 4 = seminal vesicle; 5 = ampullary gland of vas deferens; 6 = vas deferens; 7 = Cowper's gland; *B* = bladder; *C* = clitoris; *P* = penis; *U* = uterus; *V* = vagina.

those of the prostate. They are lined by a simple cubical epithelium composed of small cells. Each alveolus is surrounded by a thin sheath of connective tissue. The stroma, of which there is very little, and the vessels occupy the interstices between the alveoli.

The seminal vesicles (Text-fig. 1*b*) are large, spirally coiled, and lobulated around the circumference of the spiral. The bases taper gradually (Text-fig. 2*a–g*) and run immediately dorsal to the ampullary glands. Posterior to the neck of the bladder each seminal vesicle is continued within the sheath

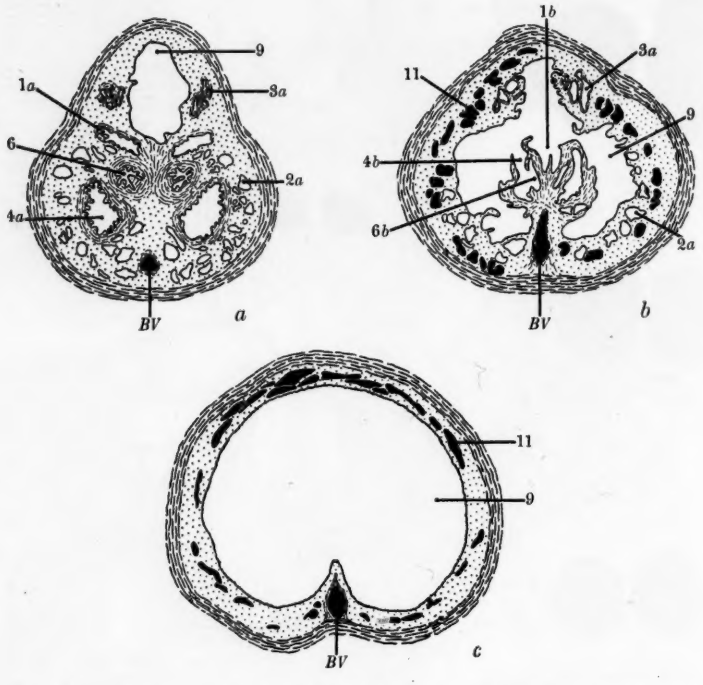


Text-fig. 2. Semi-diagrammatic scale drawings of a series of transverse sections of the prostate and associated organs of the male *Mastomys*. Figures *a* to *g* are arranged in order from anterior to posterior but the sections figured are selected, not equidistant. × 4·4. Key to guide letters: 1*a* = duct of anterior lobe of prostate; 2*a* = duct of dorsal lobe of prostate; 3*a* = duct of ventral lobe of prostate; 4*a* = duct of seminal vesicle; 5*a* = duct of ampullary gland of vas deferens; 8 = ureter; 9 = urethra; 10 = muscular sheath of urethra; other guide letters as in Text-fig. 1.

of urethral musculature as a small duct, oval in transverse section, running dorso-laterally and close to the vas deferens (Text-figs. 2*f, g* and 3*a*). The openings of the vas deferens and of the duct of the seminal vesicle into the urethra are continuous with each other (Text-fig. 3*b*). The dense colloid

secretion which fills the seminal vesicles and their ducts is intensely eosinophil, thus resembling that of the ampullary glands and sharply contrasting with that of the prostate.

The prostate gland is divided into three pairs of lobes: anterior, dorsal and ventral (Text-fig. 1 *b*). The anterior lobes are closely applied to the inner margins of the coiled seminal vesicles. The large branching tubules of which



Text-fig. 3. Semi-diagrammatic scale drawings of a series of three transverse sections of the urethra of the male *Mastomys*. Figure *a* is through the urinary region and associated ducts, figure *b* through the openings of the vasa deferentia and ducts into the urethra, and figure *c* through the urinogenital urethra slightly more posteriorly. ×16. Key to guide letters: 1*b* = opening of duct of anterior lobe of prostate; 4*b* = opening of duct of seminal vesicle; 6*b* = opening of vas deferens; 11 = urethral gland; *BV* = blood vessel. Other guide letters as in Text-figs. 1 and 2.

they are formed (Pl. 1, fig. 2) are distended with colloid secretion, which is only faintly stained with eosin. These tubules are lined by a simple cubical or low columnar epithelium which is thrown into folds that project from the wall into the lumen. The folds appear as simple finger-shaped processes in transverse sections. The epithelial cells are small, though slightly larger than those of the alveoli of the ampullary glands. The stromal sheath around each tubule

is much thicker than is the case in the ampullary glands. Each anterior lobe is provided with a single duct running dorsally to the ampullary gland and laterally to the seminal vesicle (Text-fig. 2). Posterior to the neck of the bladder this duct passes down laterally to the vas deferens to occupy a position immediately ventral to it and dorso-lateral to the urethra (Text-fig. 3*a*). The ducts from the two sides open together in the middle line into the urethra (Text-fig. 3*b*), at the same level as, but median to, the openings of the ducts of the seminal vesicles and vasa deferentia. All these three pairs of ducts open on the ventral surface of a ridge which projects from the dorsal wall of the urinogenital urethra, opposite the ventral opening into it of the urinary urethra.

The dorsal lobes of the prostate (Text-figs. 1*b*, 2) closely envelope dorsally and laterally the bases of the seminal vesicles and the muscular sheath of the urinogenital ducts. These lobes are fused in the dorsal middle line and are thickened dorso-laterally (Text-figs. 2*b–g*), where they form a projecting keel of tissue on each side which extends posteriorly some distance behind the beginning of the urinogenital urethra. The tubules composing these lobes (Pl. 1, fig. 3) are lined by a simple cubical epithelium of small cells, intermediate in size between those of the ampullary glands and anterior lobes of the prostate. Occasional folds of epithelium project into the lumen, as in the tubules of the anterior lobes. The colloid distending the tubules is not eosinophil. There is very little stroma and the connective tissue sheaths of the individual tubules are very thin. Numerous ducts from these lobes are included within the muscular sheath and open separately in the dorsal and lateral walls of the anterior end of the urinogenital urethra (Text-figs. 2*f*, *g*, 3*a*, *b*). All the ducts pass in around the anterior margin of the muscular sheath, none penetrating it posteriorly.

The ventral lobes of the prostate (Text-figs. 1*b*, 2) lie posteriorly and laterally to the bladder. Their anterior margins are widely separated by the bladder but their posterior margins meet behind it in the middle (Text-fig. 2*g*). The tubules (Pl. 1, fig. 4) are lined by a simple columnar epithelium the individual cells of which are much larger than those in the other lobes. The nuclei of these cells are also larger and the cytoplasm exhibits a characteristic zonation, that next to the lumen being dense and more readily stained, with a wider vacuolated and less readily stained zone between it and the basal region in which the nuclei are situated. Some of the cells and their nuclei are hypertrophied and are larger than their neighbours. The walls of the tubules are much folded, thus complicating the lumen. The connective tissue sheaths of the tubules are thin and the tubules are so closely packed as to leave little room for stroma. The secretion filling the tubules stains pink with eosin and is often granular. Three ducts from each lobe run down on either side of the posterior surface of the neck of the bladder and enter the muscular sheath with the urethra, running laterally to it (Text-figs. 2*f*, *g*, 3*a*). They open into the urethra ventro-laterally where the urinary region joins the urinogenital region (Text-fig. 3*b*).

The male urethra may be divided into three distinct regions. The first or urinary region (Text-fig. 3*a*) extends from the cervix of the bladder to the junction with the vasa deferentia and the prostatic and vesicular ducts. No glands are associated with the urethra in this region. It measures approximately 6 mm. long and its lumen is only 1 mm. in greatest diameter. The second region (Text-fig. 3*c*) extends from the openings of the vasa deferentia to the root of the penis, being approximately 11·5 mm. long with an internal diameter of 2·5 mm. This urinogenital region is provided with urethral glands and a well-developed musculature. A large urethral sinus with glandular walls, similar to that described in the mouse by Hall (1936), opens out of the urethra in the bulb of the penis. The third or penile region of the urethra is devoid of glands.

Cowper's glands (Text-fig. 1*b*) are a pair of large ovoid bodies, resembling those of the mouse, situated on each side of the rectum posterior to the pubic symphysis. Their ducts run into the bulb of the penis close to its attachment to the pubes.

IV. THE PROSTATE OF THE FEMALE

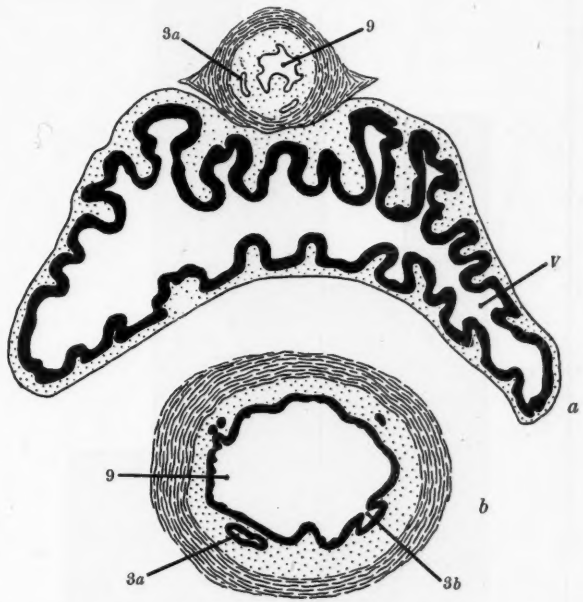
The prostate of the female *Mastomys* consists of one pair of lobes lying posterior to, and on each side of, the bladder (Text-fig. 1*a*). Dorsally these lobes are separated by the urethra and vagina. They are roughly triangular in shape. The female prostate thus resembles the ventral lobes of the prostate of the male in shape and position, and differs in these respects from both the anterior and dorsal lobes (Text-fig. 1).

Histologically the female prostate (Pl. 1, fig. 5) resembles the ventral lobes much more closely than any of the other glands associated with the genital tract of the male. The alveoli are lined by a simple low columnar epithelium, the individual cells of which are of comparable size to those of the ventral lobes of the male. They differ from them in that the differentiation of their cytoplasm into three zones, so conspicuous in the male, is either absent or barely discernible. Korenchevsky (1937) found that this zonation of the cytoplasm was characteristic of the hypertrophied prostates of female mice treated with male hormones, and he stresses the significance of this fact in establishing the homology of the organ with the ventral lobes of the male. Since the prostate of the female *Mastomys* may be nearly as large as the ventral lobes of the male and appears to be actively secretory, the apparent absence of zonation cannot be attributed to a rudimentary condition of the organ. The walls of the alveoli are deeply folded and, since less secretion is contained in them, their lumina appear even more complicated than in the male. The thin connective tissue sheath of the alveoli and the small amount of stroma present are further points of resemblance with the ventral lobes of the male.

A single duct from each lobe runs down the posterior surface of the neck of the bladder and then dorso-laterally to the urethra, within its musculature

(Text-fig. 4*a*). These ducts open into the urethra about 8·0 mm. behind the neck of the bladder. The openings (Text-fig. 4*b*) are in the dorso-lateral wall of the urethra and are situated on each side of a median ridge or prominence which projects into the urethral lumen dorsally in this region. Thus the ducts pursue, in relation to the female urethra, a precisely similar course to those of the ventral lobes of the male in relation to the urinary region of the urethra.

It may be concluded that both the anatomical and histological evidence indicates the ventral lobes of the prostate of the male as the homologue of the prostate of the female *Mastomys*.

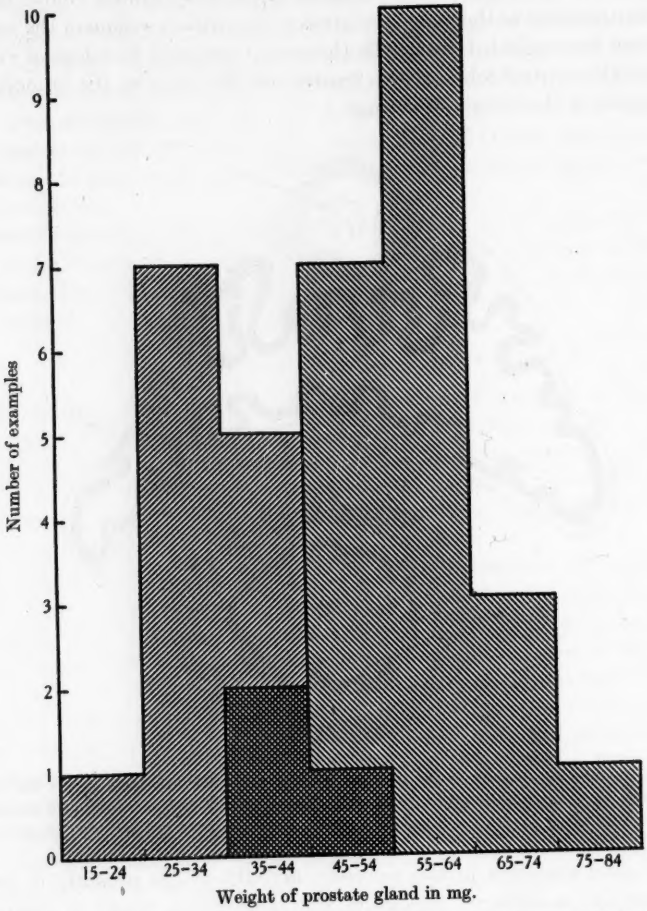


Text-fig. 4. Semi-diagrammatic scale drawings of transverse sections through (*a*) the urethra, prostatic ducts and vagina (× 20), and (*b*) the opening (=3*b*) of one of the prostatic ducts into the urethra (× 53) in the female *Mastomys*. Guide letters as in previous figures.

Individual variation in the secretory activity of the prostate in females and variation in different regions of the same gland render it difficult to determine histologically whether a regular sequence of changes, correlated with the oestrous cycle, occurs. Comparison of the weights of the glands in adult females without corpora lutea in the ovaries with those of females in which functional corpora lutea were present shows a significant difference. The average weight of the prostate in fourteen females without corpora lutea was 33·6 mg., and in twenty-three females with corpora lutea it was 57·3 mg. Frequency polygons of the distribution of the weights of the prostates in the

two groups are superimposed in Text-fig. 5. It can be seen that there is scarcely any overlap.

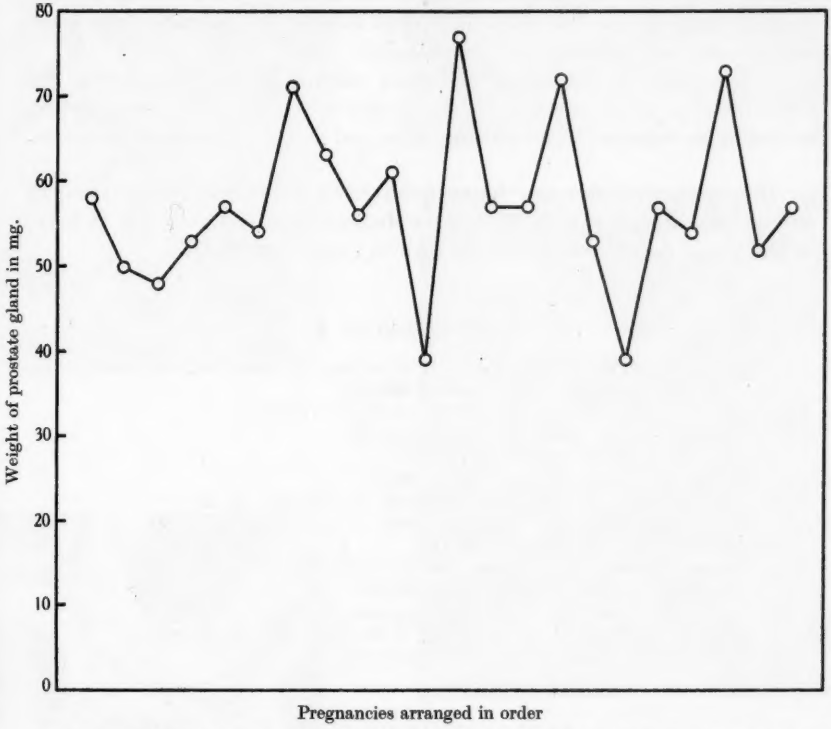
All but one of the animals with corpora lutea in the ovaries were pregnant and this one had recently aborted. The twenty-two pregnant animals ranged



Text-fig. 5. Frequency polygons of the distribution of weights of prostate in fourteen adult females without corpora lutea (left polygon) and of twenty-three females with recent corpora lutea in the ovaries (right polygon) superimposed.

from stages with fertilized ova in the stage of two pronuclei to approximately full-term. The weights of the prostates in these animals, arranged in sequence according to the stage of the contained embryos, are represented graphically in Text-fig. 6. It can be seen that the weight of the prostate tends to remain

relatively constant throughout gestation, the observed extremes being in the nature of random variations about the mean rather than points on a regular growth curve reaching a defined maximum. It may be concluded therefore that there is marked hypertrophy of the prostate of the female *Mastomys* during the luteal phase of the cycle and that this hypertrophy is completed shortly after ovulation. The hypertrophy is due, in part at least, to metaplasia



Text-fig. 6. Graphical representation of the weights of the prostate of twenty-two pregnant females arranged in order of stage of gestation, the earliest stage being on the left and the latest on the right.

of the epithelial cells during oestrus. Mitoses in the epithelial cells are fairly numerous during oestrus prior to ovulation, and are more numerous immediately after ovulation, then decreasing and disappearing very rapidly. At all other stages of the cycle mitotic figures in the epithelial cells of the prostate are rare.

V. SUMMARY

A large prostate gland is present in all normal females of the multimammate mouse, *Mastomys erythroleucus* Temm. It consists of a pair of lobes, each provided with a duct opening into the urethra.

The anatomy and histology of the prostate and associated glands in the male are described for comparison. Both anatomically and histologically the prostate of the female resembles the ventral lobes of the prostate of the male, with which it is concluded to be homologous.

The prostate of the female undergoes marked hypertrophy during the luteal phase of the oestrous cycle. The hypertrophy occurs at oestrus, accompanied by metaplasia of the epithelial cells, and persists throughout gestation.

The expenses of this research were defrayed by grants from the Government Grant Committee (to F. W. R. B.) and the Medical Research Fund (to D. H. S. D.) of the Royal Society, for which we wish to express our thanks.

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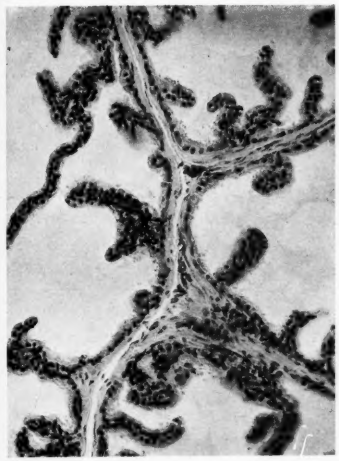
EXPLANATION OF PLATE 1

Fig. 1. Photomicrograph of a section of the ampullary gland of the vas deferens. × 100.
Fig. 2. Photomicrograph of a section of the anterior lobe of the prostate of the male. × 100.
Fig. 3. Photomicrograph of a section of the dorsal lobe of the prostate of the male. × 100.
Fig. 4. Photomicrograph of a section of the ventral lobe of the prostate of the male. × 100.
Fig. 5. Photomicrograph of a section of the prostate of the female during mid-pregnancy. × 100.



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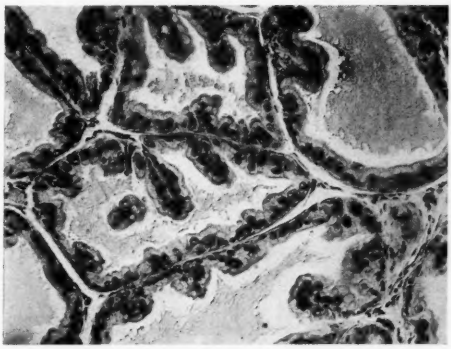

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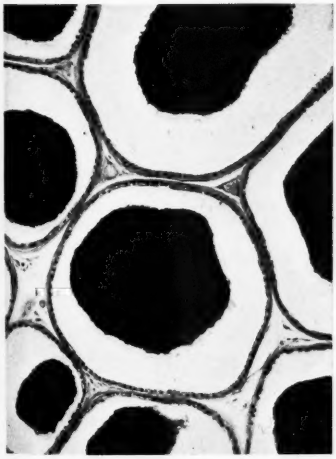
Fig. 5.



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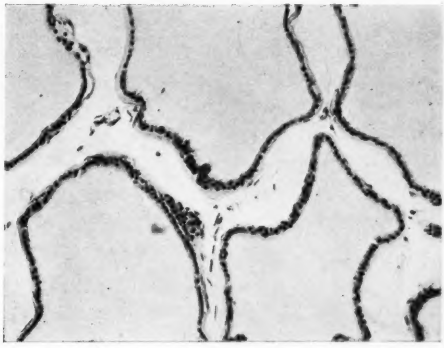


Fig. 3.

UNIV. OF MICH.

SOME OBSERVATIONS ON THE CARDIO-VASCULAR SYSTEM IN THE VIABLE FOETAL LAMB

By K. J. FRANKLIN, A. E. BARCLAY
AND M. M. L. PRICHARD

From the Nuffield Institute for Medical Research, Oxford

During the seasons 1937–8 and 1938–9 we co-operated with Sir Joseph Barcroft and Dr D. H. Barron in X-ray cinematographic studies that had as their initial object the determination of the time of functional closure of the ductus arteriosus. These studies led to observations upon the foetal circulation as a whole, and of them we have published a preliminary account (Barclay *et al.* 1939), together with notes on the identification of the ductus arteriosus in radiographs. An account of the functional closure of the ductus, comprising evidence from blood-gas analyses and from X-ray cinematographic records, is to be published elsewhere, and there will also be presented at some future date a detailed analysis of all our radiological data.

We had hoped to co-operate in a third season's programme with the Cambridge workers, but this hope, much to our regret, has been frustrated by the War. We have, therefore, done two things by ourselves. The first of these was to initiate a comprehensive research into the literature upon the foetal cardio-vascular system and circulation. This research provided us with a series of questions, both anatomical and physiological, to which, as our second objective of this season, we have attempted to find answers.

In the present paper we wish to deal mainly with the more anatomical questions, and with certain other details of structure that are of importance in the viable foetal lamb, for this animal is the one that has been, and probably will be, most used in experimental studies of the foetal circulation.

The radiological techniques that we have employed have included both direct and indirect X-ray cinematography (the apparatus is described elsewhere—Barclay *et al.* 1940), and ordinary radiography. We have in every instance made injections of contrast medium (the substances used being thorotrast, perabrodil or diodrast, uroselectan, lipiodol, lipiodol emulsified with normal saline, and—for post-mortem work only—1 in 1 sodium iodide solution), varying the medium and the amount injected according to the requirements of the particular case.

It is pertinent, at the outset, to point out that the living anatomy (e.g. the size and position of the liver and other viscera, and the calibre of the vessels) has in many cases differed from the post-mortem anatomy, on which the observations made by earlier workers have been based.

The features that we wish to discuss are:

I. The placenta and the umbilical veins.

II. The liver, umbilical vein, portal vein, ductus venosus, and hepatic veins. The junctions of the ductus venosus and hepatic veins with the vena cava.

III. The intervenous tubercle.

IV. The valve of the foramen ovale.

V. The absence of valves of the inferior vena cava and coronary sinus.

VI. The azygos venous system.

VII. The contractions of the heart chambers.

We do not propose to treat of the ductus arteriosus, as its anatomy has already been very adequately dealt with at Cambridge and elsewhere (see von Hayek, 1935; Boyd, 1937; Barcroft, 1938).

I. The placenta in the sheep is cotyledonous.

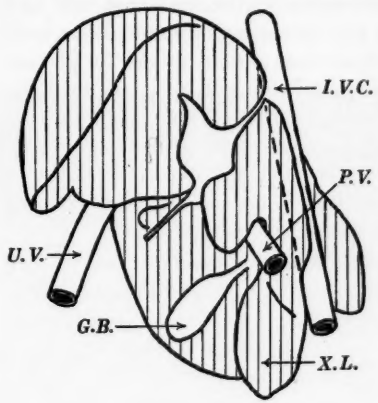
The venous blood from the cotyledons is collected into two large veins that remain separate within the umbilical cord. It is not easy to determine the length of the cord because of its retractive power, but it is of the order of 20–25 cm. and it does not show the wide variations that occur in Man. So far as our observations go, the main umbilical veins—the only ones that we have examined in detail—are valveless in the sheep. The twin vessels are about 4–6 mm. in external diameter within the functioning umbilical cord. They are very responsive to some forms of mechanical stimulation and may contract down to half their initial width for some distance if they are freed from their surroundings. On the other hand, their width is not usually materially altered if sharp hypodermic needles are introduced into them through the intact outer covering of the cord. The number of injections made by our previous collaborators and ourselves into umbilical veins is fairly large, and in every instance the choice between the two veins has been an arbitrary one. Inasmuch as the radiographs of the courses of the injections have been very similar, we may provisionally assume that there is no differential distribution within the foetus of the blood from the two umbilical veins.

Where they pass through the skin of the foetus, the two veins may be described as right and left, i.e. they enter in the same transverse plane with respect to the long axis of the body, and their shadows are superimposed in the position that we have ordinarily used for X-ray cinematography, i.e. the lateral one. Either at their entry into the foetus, or within a distance of 1·5 cm. nearer the liver, the veins fuse externally along their surface of contact. They pass through the skin almost perpendicularly to the external surface but, once through it, they bend rostrally (sometimes very sharply), and they also incline, viewed sagittally, about 10–15° to the right from the midline. There must be a distinct difference between the extra- and intra-foetal portions of the veins, for we have never seen a contraction spread from the former to the latter, and in fixed specimens the former is contracted and thick-walled, the latter wide and thin-walled.

About 2 cm. proximal to the point at which they pass through the skin of the foetus, the umbilical veins fuse internally, the "party-wall" between them

terminating in a crescentic edge. About 2 cm. proximal to this internal junction, the single vessel formed by the union reaches the border of the liver (in fixed specimens the mid-line of the vein is now about 1·3 cm. to the right of the sagittal mid-line of the body), and thereafter it passes for about another 1 cm. within a fossa before actually penetrating into the liver substance itself.

II (Figs. 1–3). In the living foetus, in the sagittal position, a small portion only of the liver lies to the left of the mid-line of the body. The viscus as a whole is kept over to the right by certain attachments and also by a greatly



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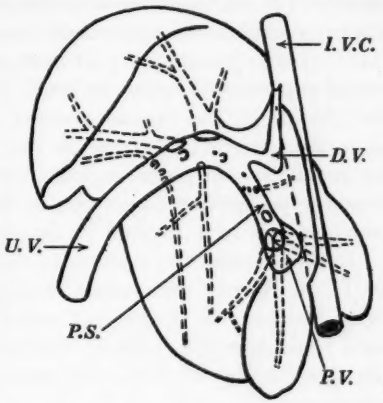

Fig. 2.

Fig. 1. Left lateral view of the fixed liver of a foetal lamb. *I.V.C.* inferior vena cava, rather strongly contracted by the fixative. *P.V.* portal vein. *U.V.* umbilical vein. *G.B.* gall bladder. *X.L.* what is referred to in the text as the *x* lobe. Centimetre scale.

Fig. 2. The same liver as in Fig. 1 with small superficial portions removed and the umbilical vein, ductus venosus, "portal sinus", and portal vein opened up. Interrupted lines mark the approximate courses of certain venous branches going to the liver substance; four of them have a common exit at the lowest part of the "portal sinus". *I.V.C.* inferior vena cava. *D.V.* ductus venosus. *P.V.* portal vein. *P.S.* "portal sinus". *U.V.* umbilical vein. Centimetre scale.

dilated stomach that, apparently, contains amniotic fluid.[1] In consequence, we have found sagittal X-ray records of little value as guides to the distribution of the different vascular systems within the liver. In passing, it may be noted that this organ alters rather considerably in position and in shape post-mortem. In a full-term foetus the longitudinal axis of the inferior vena cava, at its exit from the liver, is about 1·6 cm. to the right of the sagittal mid-line of the body.

The liver is not easy to describe in words, but its general features can be grasped by reference to the two drawings of a fixed specimen (Figs. 1, 2).

[1] Incidentally, the rectum may contain large amounts of formed scybala.

A noticeable point is the superficial position of the umbilical vein and "portal sinus". In Fig. 2 are shown the umbilical and portal veins and some of the larger branches passing from them to the liver substance. What we have, for brevity, designated as the *x* lobe of the liver is a constant feature in radiographs of the living lamb.

For over three centuries, and in particular since the publication of Glisson's book on the anatomy of the liver (1654), there have been conflicting views as to which of the afferent veins should be regarded as branches of the portal vein, and which as branches of the umbilical vein. In addition, some have regarded the ductus venosus as the continuation of the umbilical vein and others have denied this. The main reason for the variety of opinions has been that they were based upon post-mortem dissections and injections, for physiological experiments upon the foetal circulation were not initiated until the first decade of the present century (Pohlman, 1909). Among the earlier anatomists, Bertin (1753) was the one who gave the most accurate account of the umbilical and portal venous distributions, but his injections failed to show the progressive diminution in calibre that is typical of the *functioning* umbilical vein as it gives off its branches within the liver (Fig. 3).

In the previous two seasons, in co-operation with Sir Joseph Barcroft and Dr D. H. Barron, we made a number of injections into umbilical veins of living foetuses. In the present season we have, on several occasions, made *intra vitam* injections into an umbilical vein and a large mesenteric vein in the same foetus. We have also made immediate post-mortem injections for comparison.

The results show, in the first place, that the calibres of the dead and living vessels are fundamentally different, for the dead veins fill to their maxima all along their length and give an entirely different picture from that shown, for instance, in Fig. 3. The *intra vitam* injections also give definite information about the dual venous inflow into the liver. The umbilical vein flow is of far greater importance than the relatively small portal flow, for it is distributed through branches both of the umbilical vein proper and also of the so-called "portal sinus". The portal vein flow, on the other hand, passes only to the *x* lobe and to the remaining lower right portion of the liver, and these parts also derive some of their supply from the umbilical vein. We have never seen portal vein blood pass along the "portal sinus", and the term is, therefore, in our opinion, a misnomer in an account of the functioning foetal cardiovascular system.

The branches of the umbilical vein supply a large portion of the liver, as Fabricius (see his Figures) and Bertin (*loc. cit.*) both realized. As it gives off these branches, the living vein decreases progressively in calibre until, at its termination, it is of a width not greatly exceeding that of the ductus venosus (Fig. 3). The orifices of some of the branches are set facing the main umbilical stream. In X-ray cinematographic records, taken in our usual lateral position (Fig. 3), we can generally see three branches of the umbilical vein going up-

wards in the radiograph (=upwards and to the left in the actual liver) and two downwards (=downwards and to the right in the actual liver). The third downward branch is the "portal sinus". These upward and downward branches of the umbilical vein are not so constant in their direction and distribution as they are in their number, nor do they represent the total outflow, for some other branches pass off in such a way as to be indistinguishable in a lateral radiograph.

The ductus venosus at its origin may show a lip such as was mentioned by Bichat (1801), who thought that it would prevent portal vein blood from passing into the ductus after birth. The ductus venosus in the living viable lamb always exhibits the sharp kink and narrowing that is seen in Fig. 3, though

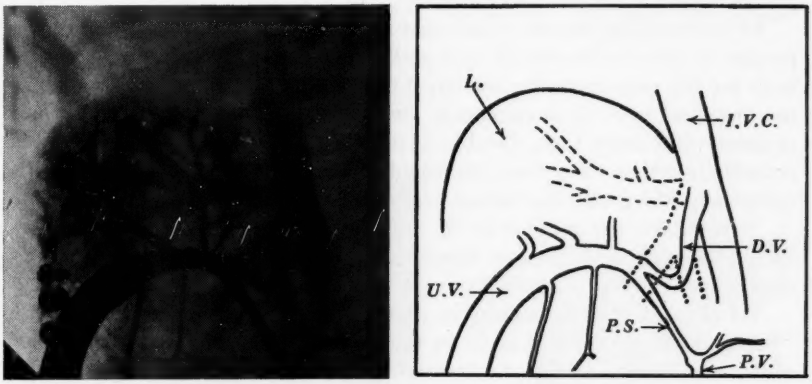

Fig. 3. Part of a lateral radiograph of a living foetal lamb to show the relations of various liver vessels. The centimetre scale gives the dimensions in the radiograph, i.e. dimensions slightly larger than the true ones. *L.* liver. *I.V.C.* inferior vena cava. *D.V.* ductus venosus. *U.V.* umbilical vein. *P.S.* "portal sinus". *P.V.* portal vein. Interrupted lines, large left hepatic vein. Dotted lines, large right hepatic vein.

thereafter it widens again before its entry into the inferior vena cava. It is possible that this kink becomes more marked if the liver shrinks with the post-natal cessation of the umbilical venous flow, but we have no direct records to prove or disprove the point, and must now reserve the problem for solution during another season.

It is of interest to note, in the lateral radiographs, that the calibre of the narrow portion of the functioning ductus venosus is about one-third that of the umbilical vein before the latter has given off its first branches. Hence, if the cross-sections of both vessels are circular, that of the ductus must be about nine times smaller in area than that of the umbilical vein.

In its hepatic portion the inferior vena cava is first joined by a variable number of veins draining the lower right portion of the liver (including the

x lobe); these vessels are irregular in size, though one or more may be fairly large. The blood that they collect appears to be derived largely from the portal vein. More rostrally, the vena cava is joined by three vessels of a calibre not much smaller than its own. They are the two main hepatic veins and the ductus venosus, and a section across the level of their junction reveals the vena cava on the dorsal aspect, and ventral to it, from left to right, the ductus venosus and the two large hepatic veins. The four vessels are separated at their union by thin membranous septa that must yield freely to permit variations in the blood flow through the individual channels. The ductus venosus cannot be said to enter the neighbouring hepatic vein, as it has been said by some to enter the corresponding vein in the human. The tops of the septa between the four vessels are at approximately the same level, though there are variations in this respect in different lambs.

Of the two large hepatic veins, that next to the ductus venosus drains the portion of liver to the left of, and rostral to, the umbilical vein. The other large hepatic vein drains the portion of the liver to the right of, and caudal to, the umbilical vein. It is commonly formed by the union of two, sometimes of three, veins about 1 cm. distal to its union with the vena cava (Fig. 3). It is potentially rather wider than the neighbouring hepatic vein, as that vessel is potentially wider than the ductus venosus at its termination.

It is outside our purpose in the present paper to detail the distribution within the heart of the various streams of blood that form the thoracic inferior caval flow.

III (Figs. 4–6). The passage in which Richard Lower first described the "tuberculum" or swelling is to be found on pp. 48–50 of his *Tractatus de corde*, etc. (1669). It may be translated (Franklin, 1932) as follows: "Just before the threshold of the right auricle, that is to say, where the ascending vena cava joins the descending[1] and prepares to discharge into the auricle of the Heart, a certain small swelling is formed by underlying fatty tissue. This swelling is well worth attention. Its interposition at this point causes the blood which falls down the descending vein to be diverted into the auricle, when it would otherwise have continued on down into the ascending vein and would have caused great hindrance and slowing to the blood flow up through that vein into the heart." Lower, erroneously, considered that the swelling was more marked in man than in pronograde mammals, and his first figure, which pictures it in man, is obviously incorrect. His second figure (reproduced as Fig. 4 of the present paper), pictures the swelling as it occurs "in quadrupeds, such as the sheep, dog, horse and ox", but there is no indication in Lower's text or legend as to which of these animals provided the material for the illustration. From our own experience of various species we should imagine that he drew from a lamb's heart (cf. Fig. 6).

[1] This division of the single vena cava of the earlier writers (including William Harvey) into two portions, and its separation from the right auricle, was a useful advance in terminology. We are not here claiming that Lower introduced the change—further research would be necessary to decide the point.

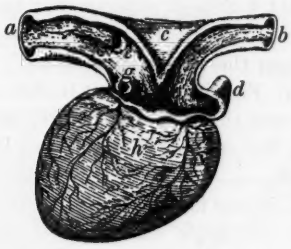

Fig. 4. The intervenous tubercle, as pictured by Lower (1669, Pl. I, fig. 2). *a*, trunk of the ascending vena cava. *b*, trunk of the descending vena cava. *c*, the swelling (*tuberculum*) separating the two veins. *d*, right auricle. *e*, foramen ovale. *g*, coronary vein. *h*, heart [i.e. the ventricles].

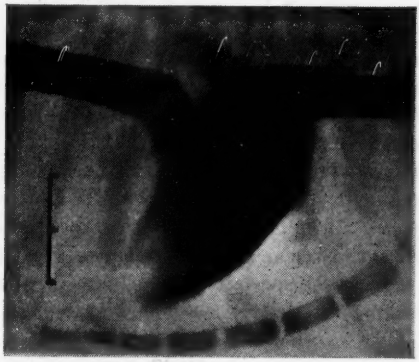

Fig. 5.

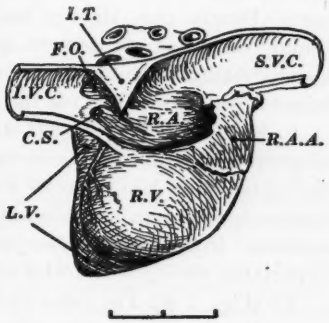

Fig. 6.

Fig. 5. Lateral radiograph of the heart and great veins, shown up by injected contrast medium, in a living foetal lamb. The intervenous tubercle is clearly seen between the two venae cavae. The centimetre scale gives the dimensions in the radiograph, which are slightly larger than the true ones.

Fig. 6. Drawing of the heart and terminations of the great veins, as seen from the right side in a viable foetal lamb, to show the intervenous tubercle. The venae cavae and right atrium have been opened up, as in Fig. 4. *I.V.C.* inferior vena cava. *S.V.C.* superior vena cava. *R.A.* right atrium. *R.A.A.* auricle of right atrium. *R.V.* right ventricle. *L.V.* left ventricle. *C.S.* opening of coronary sinus. *F.O.* foramen ovale. *I.T.* the intervenous tubercle, above which are some right pulmonary veins and arteries in section. Centimetre scale.

Since Lower's time, his finding has been variously accepted or denied (see Walmsley, 1929), or doubt has been expressed as to what exactly he meant (e.g. by Ziegenspeck, 1884, p. 599). In addition, the word "torus" has been suggested as a substitute for the word "tuberculum", though the meanings of the two words are similar. Finally, though it is obvious from Lower's original text and figures that he was thinking particularly of the tubercle in relation to the foetal circulation, the swelling has not been very seriously considered by writers on that subject.

We ourselves noticed, in our lateral X-ray cinematographic records of the functioning foetal heart, an angle that was formed between the superior and inferior caval streams (Fig. 5), and that resembled very closely the angle in Lower's figure. Post-mortem examination of foetal hearts (see, e.g. Fig. 6) showed us quite clearly that the angle was, in fact, caused by Lower's tubercle and that his description (except for his references to the human heart) was a correct one.

Though Lower was, we believe, the first to *illustrate* the anatomical basis for the division of the inferior caval stream in the foetus (he placed the foramen ovale, quite correctly, in the ascending vena cava below his tuberculum intervenosum), a careful reading of Harvey's account of the foetal circulation (1628) suggests that he, too, had a correct appreciation of the anatomy of the parts. Despite this, Harvey has persistently been misquoted, up to and including the twentieth century, as saying that the inferior and superior caval blood streams of the foetus mix within the right atrium, and that part of the mixed blood then passes *via* the foramen ovale into the left atrium.

C. F. Wolff (1776, pp. 395–6) thought that he was the first to locate the foramen ovale correctly, but it is obvious that the English anatomist, Lower, had anticipated him by over a century. The tuberculum intervenosum has seemed to many an insignificant structure by which to remember so great a scientist. Our findings show that, in certain species, it is of considerable importance during both foetal and adult life.

IV (Figs. 7–8). The valve of the foramen ovale in the foetal lamb is a tube rather than a valve in the ordinary sense of the word. Wolff (1776) was the first to note such tubular valves, the species in which he described them being the human and the bovine. Kilian (1826), who was Wolff's scientific descendant so far as the foetal cardio-vascular system was concerned, was the first to describe the tubular valve of the foetal lamb, though his drawing of a specimen (see his Pl. V, fig. 4) is lacking in clarity. Rowland (1894) described various forms of the valve as found in the adult sheep, and noted a similarity to the atrio-ventricular valves. We ourselves, after dissecting a number of foetal hearts, have been able to recognize the functioning valve in both lateral and sagittal X-ray cinematographic records.

It is easier to demonstrate the tube than to describe it. It is of appreciable length and very wide at its origin, though its diameter diminishes markedly in the first half of its length (Fig. 7). Thereafter it may show a slight increase

in diameter, followed by a second decrease, or it may retain a uniform diameter throughout the second half of its course (as in Fig. 7). Near its free end it very often shows fenestrations, and in all the specimens that we have seen there were very fine "cords"[1] running from this free edge to the atrial wall.

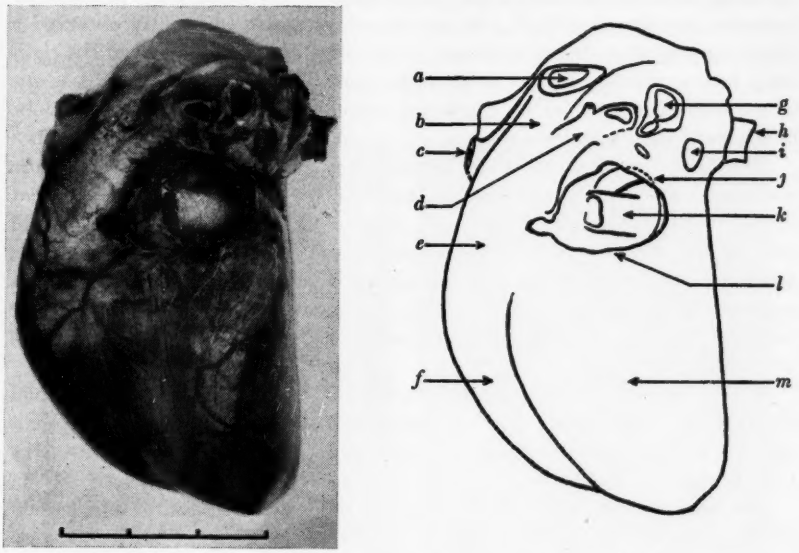

Fig. 7. Photograph, taken from the left side, of the heart of a viable foetal lamb in which the tubular valve of the foramen ovale was found fully open on dissection. The outer wall of the left atrium has been removed. Centimetre scale. *a*, the descending aorta distal to its union with *b*, the ductus arteriosus. *c*, the brachio-cephalic artery. *d*, the left pulmonary artery. *e*, the pulmonary trunk. *f*, the right ventricle. *g*, the left main bronchus. *h*, the thoracic inferior vena cava. *i*, a large left pulmonary vein. *j*, the edge of the circular recess in the left atrium in which lie the valve of the foramen ovale and the entries of certain small upper right pulmonary veins. *k*, the tubular valve of the foramen ovale; the free end of the tube shows up dark in the photograph. *l*, the line of exsection of the outer wall of the left atrium. *m*, the left ventricle.

These "cords" appear to be too long to have much restraining influence. The tube is attached medially along a small area of its total circumference. Its direction in the normal lie of the functioning foetal heart is more or less parallel to the line of the vertebral column. Its first portion lies in a circular

[1] Mr E. H. Leach, of the University Department of Physiology, Oxford, has very kindly sectioned such a "cord", taken from the specimen pictured in Fig. 7. He finds that the "cord" consists of a few cardiac muscle fibres, encased in a wider layer of collagen fibres. The former are not identifiable as Purkinje fibres, which is not surprising, as such fibres only develop well in the adult. "As the collagen fibres would presumably prevent the effective contraction of the few small muscle fibres, the latter could be looked upon as conductile." The presence of such conductile cords could explain how the impulse to contract simultaneously with the atria as a whole is conveyed to the musculature of the valve (see Barclay & Franklin, 1938).

recess common to it and to the entries of three or more medium-sized pulmonary veins draining the upper part of the right lung. This is, perhaps, of some importance with regard to the post-natal closure of the valve, for we cannot see from the structure that this closure can be anything but mechanical, and the flow from the upper part of the right lung presumably increases greatly after birth. In our records we have constantly observed a fairly copious pre-natal arterial and venous flow in the lower part of the right lung, but no very great flow in its upper part. The sheep, incidentally, is one of the mammals in which an eparterial bronchus supplies the uppermost lobe of the right lung with its air-entry.

We have some evidence that the valve may close temporarily even before respiration has commenced, and we can conceive its doing so to some extent during atrial contraction; this was, incidentally, first suggested by Wolff (loc. cit. pp. 392–3). But, on the anatomical evidence, we would suppose its main functional closure to result from increased pulmonary venous return accompanying the onset of respiration. This increased return *post partum* may normally depend upon functional closure or partial closure of the ductus arteriosus.

Fig. 8 consists of stereoscopic photographs of the valve from a second foetal heart in the nearly fully open and almost shut positions. The latter is the one in which the valve was found on dissection, and the free end of the tube is partially tucked in.

We hope at some future date to publish the results of a comparative study of the valve of the foramen ovale in several species, for we have found interesting resemblances and differences between them in respect of this structure.

V. Walmsley (1929) states that the valves of the inferior vena cava and coronary sinus "are absent in most Carnivora, Cetacea, and Chiroptera, and in some other forms, and in many species one or other may be wanting". We have not found them in any of the foetal lamb hearts that we have examined, and in this connexion we should like to adduce a point that seems to have escaped the notice of previous writers during the last century and a half. The most prevalent hypothesis about the course of the foetal circulation from 1774 onwards until well into the present century was that of Sabatier, or a modification of it. This hypothesis, however, depended upon Sabatier's concept of the function of the Eustachian valve. Hence, it cannot, on the comparative anatomical findings, be basically true for all mammals, and it is certainly not true in this sense for the sheep, the animal with which we are here more particularly concerned. We wish to mention this point, for our actual findings about the foetal circulation (Barclay *et al.* 1939) have some resemblance to Sabatier's schema, and we do not wish our work to be quoted in support of that schema when we are dealing with an animal in which the basis for it is lacking.

VI. The azygos venous drainage is of importance in any thorough examina-

tion of the foetal circulation, though it was passed over by Ziegenspeck (1905) when he formulated his hypothesis of this circulation. In the sheep the drainage from ten segments enters the superior vena cava, while that from sixteen segments enters the coronary sinus *via* the oblique vein of Marshall. The exit of the coronary sinus into the right atrium is correspondingly large.

VII. The contractions of the chambers of the post-natal heart within the intact thorax, as observed upon the fluorescent screen, are fundamentally different from those obtaining after the thorax has been opened. The radio-

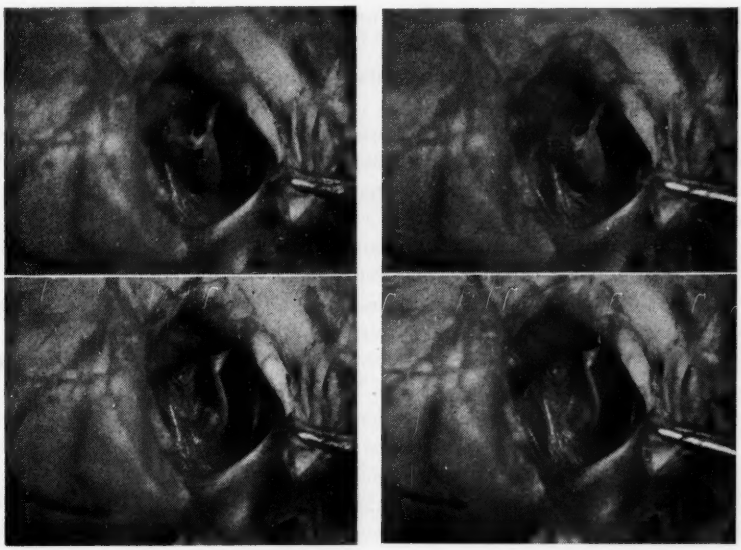

Fig. 8. Stereophotographs of another foetal lamb's heart, with the left atrium opened, to show the valve of the foramen ovale nearly fully open (above) and almost closed (below). Note the fenestrations at the free end of the tube and the "cords" passing across to the atrial wall.

logical evidence shows that, under the more normal conditions, there is no great change in the external contour of the heart but that rather the atrio-ventricular ring moves towards each end of the heart in turn as first the atria and then the ventricles contract. This evidence existed before the more modern developments of X-ray cinematography, but it has since been amply confirmed by Böhme, in a number of animals, by the use of this technique. So far as we know, however, he has never investigated the contractions in the foetus, so it is of interest to note that we have records that prove the foetal heart to behave similarly in this respect to the adult.

SUMMARY

From radiographic and X-ray cinematographic studies of the living animal, and from dissections of the dead one, we have made a number of observations about the cardio-vascular system of the viable foetal lamb. They include the following:

1. The umbilical veins are two in number, and valveless, within the cord, but they fuse within the foetus.

2. The functioning umbilical vein diminishes in calibre within the liver as it gives off its branches, so that at its termination it is of a calibre not greatly exceeding that of the ductus venosus.

3. The "portal sinus" is, functionally, an offshoot of the umbilical vein and not of the portal vein.

4. The functioning ductus venosus has a cross-section, at its narrowest point, about nine times smaller than that of the umbilical vein before the latter has given off its first branches. The ductus venosus always exhibits a marked kink in lateral radiographs.

5. The umbilical venous inflow, and not the portal one, dominates the hepatic picture while the placental circulation is continuing.

6. The outflow from the liver passes through the ductus venosus, two large upper hepatic veins, and a variable number of lower and smaller ones.

7. The intervenous tubercle of the sheep was correctly described and pictured by Richard Lower in 1669. It is visible in lateral radiographs of the living foetal lamb, and has considerable functional importance.

8. Lower also located the foramen ovale, quite correctly, in the inferior vena cava and not in the right atrium.

9. The valve of the foramen ovale in the foetal lamb is a very remarkable tubular structure, and its closure would appear to be effected mechanically. Such closure may, perhaps, occur during atrial contraction but more important is the closure that occurs *post partum* with the onset of respiration. In this connexion it may be noted that the venous return from the upper part of the right lung (this part has, apparently, no very great circulation through it before birth) enters the left atrium alongside the tubular valve of the foramen ovale.

10. The valve of the inferior vena cava (the anatomical basis of Sabatier's hypothesis of the foetal circulation) and that of the coronary sinus are not found in the viable foetal lamb.

11. The larger part of the azygos venous return enters the heart *via* the oblique vein of Marshall—a large vessel in the sheep—and the coronary sinus.

12. The heart chambers in the intact sheep foetus have been seen to contract as they do in the intact adult of various species, i.e., there is no great change in the external contour of the heart, such as occurs with the thorax open, but rather the atrio-ventricular ring moves in turn towards each end of the heart as first the atria and then the ventricles contract.

ACKNOWLEDGEMENTS

To Dr J. Trueta, to Mr J. C. Scott, F.R.C.S., and to various medical students from Oriel and other Colleges, we are indebted for much generous assistance during our operative procedures. We should like, in addition, to acknowledge the great help given to us by Mr Maurice Tuckey, our technician. To Miss A. J. Arnott we owe the excellent drawing that is reproduced in Fig. 6, and to Mr W. Chesterman the equally excellent photograph that is reproduced in Fig. 7.

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FURTHER OBSERVATIONS ON THE STRUCTURE OF HUMAN HEART-VALVES

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INTRODUCTION

Ritter *et al.* (1928) found blood vessels in 2% of what were then considered normal human heart-valves. In later observations Gross and his co-workers (1931, 1936*a*, *b*) expressed doubt as to the normality of any human heart-valve possessing blood vessels, and Gross (1937), in a final report, concluded that "blood vessels do not exist in normal valves, or if they do, they must be rare".

Aagaard (1924) has shown that no lymph vessels are present in human valves at any age period.

Human heart-valves which are the site of an inflammatory process, however, become vascularized to a greater or lesser extent by the new formation of vessels. These are derived almost entirely from atrial blood vessels and ramify for the most part in the subendothelial zone of the cusps (Darier, 1888; Gross, 1921; Harper, 1938).

Early stages of human valvulitis, in which the initial histological changes that accompany the growth of new blood vessels into the cusps can be demonstrated, are difficult to secure, for the issue in this form of the disease is rarely fatal. The present paper contains a description of an early histological reaction accompanied by new formation of blood vessels in a case of rheumatic valvulitis in the mitral valve of a child aged 8 years. I have been unable to find in the literature an account of the histology of a normal human mitral valve at this age period. To facilitate comparison, therefore, a description based on the examination of numerous longitudinal serial sections of the anterior cusp of the normal mitral valve of a child aged 9 years is also given.

Histology of the normal human mitral valve (Figs. 1, 2). The endothelium on the atrial aspect consists of a single layer of oval or rounded cells closely packed in the proximal portion of the cusp but fewer in number and more elongated towards the free margin. The scanty protoplasm appears to be agranular. At certain sites, notably in the proximal portion and on the area of contact of the cusp, there are gaps of one or more cells of this layer, and, in the subendothelium immediately subjacent to the gaps, rounded cells with the characters of endothelial cells occur. The nature and origin of the latter are doubtful but the possibility of their having differentiated from cell-elements of the subendothelium to replace desquamated endothelium seems likely.

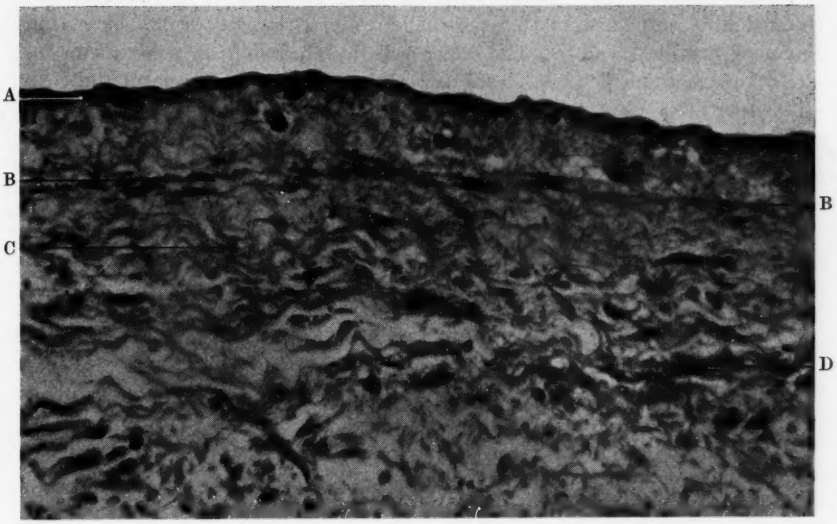

Fig. 1. Longitudinal section of the anterior cusp of the mitral valve (proximal portion) of a child aged 9 years to show the atrial endothelium and subendothelium (normal). Iron haematoxylin. A, endothelium; B, superficial elastic layer; C, collagenous reticulum; D, deep elastic layer.

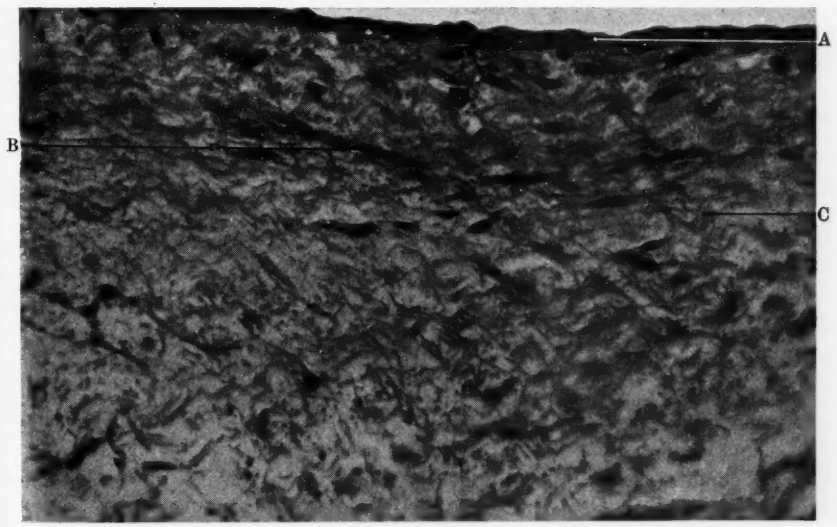

Fig. 2. Longitudinal section of the same cusp at the area of contact. Iron haematoxylin. Lettering as in Fig. 1.

The subendothelium exhibits the following features. A narrow zone lies immediately subjacent to the endothelium and is limited deeply by a thin layer of fine elastic fibres. The latter will be referred to in subsequent descriptions as the superficial elastic layer. In the middle portion of the cusp this layer becomes attenuated and the zone superficial to it relatively wider, while in the distal third it could not be distinguished. More deeply, a wider zone of closely banded collagenous fibres comprises the main mass of the subendothelium. The deep part of this zone contains an abundance of coarse elastic fibres prominent throughout the whole length of the cusp. The spiral arrangement of the elastic fibres around the collagenous bundles and their intimate relation to the central "fibrosa" is noteworthy.

Fibroblasts constitute the chief cellular element of the subendothelium. They are most abundant in the distal two-thirds of the cusp and are commonly found adjacent to the surfaces of collagenous bundles, their cell processes being often indistinguishable from the latter. The cells are spindle-shaped in profile, and are of a highly differentiated type. Smaller cells having the same general appearance are numerous in the loose connective tissue of the proximal portion of the subendothelium.

Scattered throughout the subendothelium above and below the superficial elastic layer are cells of oval or flattened outline, with small nuclei, often irregular or bilobed. Their cytoplasm is frequently vacuolated and no nucleoli could be made out. These cells have the character of histiocytes.

Proximally the subendothelium is directly related to the atrial musculature.

The central zone or "fibrosa" which affords mechanical support to the cusp is, at this age period, already composed of a dense fibrohyaline plate extending from the annulus fibrosus to the free margin. Rounded cells and a basophil fibrillated collagenous interstitial substance are its main constituents.

The ventricular endothelium presents a single layer of rounded cells with spherical nuclei. The cells are smaller and less closely packed than on the atrial aspect. The subendothelium in this region is narrow, few elastic fibres are present, and it is often difficult to distinguish it from the adjacent fibrosa. This zone is not continued into the chordae tendineae and is attenuated or even absent at their insertion.

No blood vessels or myocardial extensions were observed in any part of the cusp.

Histological reaction and new blood-vessel formation in early human valvulitis (Figs. 3, 4). The case was that of a female aged 8 years. The post-mortem findings included moderate hypertrophy of the right and left ventricles and a slight thickening with retraction of the free margin of the mitral valve. The tricuspid and arterial semilunar valves were normal to macroscopic inspection. The following description deals with the histological appearances seen in longitudinal section of the anterior cusp of the mitral valve.

The atrial endothelium is intact and the cells are larger than normal. The

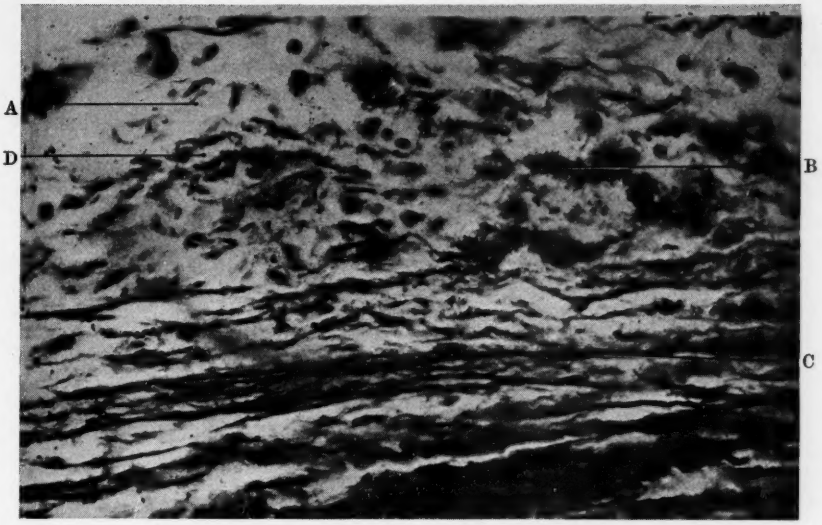

Fig. 3. Longitudinal section of the anterior cusp of the mitral valve (proximal portion just under endothelium) of a child aged 8 years (case of rheumatic valvulitis). Weigert's elastic stain. A, subendothelium; B, skein-like masses of superficial elastic layer; C, deep elastic layer; D, new capillary.

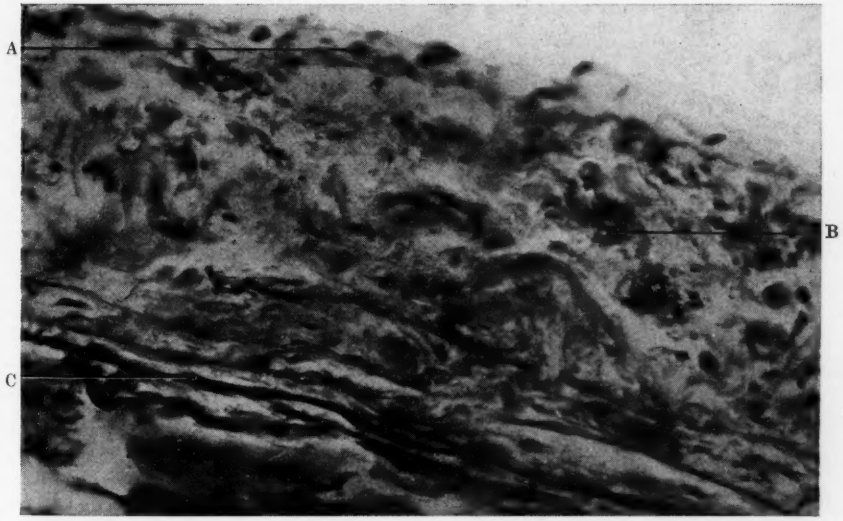

Fig. 4. Longitudinal section of the same cusp at the area of contact. Weigert's elastic stain. A, endothelium; B and C as in Fig. 3.

layer presents rather an oedematous appearance though the size of the nuclei is unchanged.

The cellular content of the atrial subendothelium is greatly altered throughout its entire surface extent and depth, the reaction being more intense in the region adjacent to the atrial myocardium and under the area of contact of the cusp. Marked swelling of the collagenous reticulum accompanies the cellular change.

New blood vessels were observed only in the proximal part of the subendothelium, and their continuity with vessels of the atrial musculature was established in serial sections. They are situated at and below the level of the superficial elastic layer. All are of capillary size, ranging from 10 to 25 μ in diameter. One of the new vessels is seen in longitudinal section in Fig. 3 D: this was the longest observed and it extended some 3·5 mm. into the cusp. It could not be traced farther than the site shown and this portion may be regarded, therefore, as the terminal part of an advancing capillary bud. The wall is composed of sparsely distributed endothelial cells only the elongated nuclei of which were demonstrable in most cases. Surrounding the capillary is an "adventitia" derived, it would appear in great part, from undifferentiated mesenchymal cells which in this region of the normal subendothelium outnumber the fibroblasts. Mitotic figures are common in cells throughout the whole depth of the subendothelium in this region.

The new blood vessels have arisen in the first instance by proliferation from vessels in the atrial muscle, but it is clear also that cells associated with their subsequent establishment in the cusp are derivatives of certain subendothelial connective tissue cells which proliferate alongside the advancing capillaries. It would be hazardous, however, to attempt from a single specimen a definition of the precise origin or role of the cells concerned in this reaction.

The elastic layers of the cusp have also undergone marked alteration. The superficial layer has disappeared except in a few places where the fibres associated with fibroblastic proliferation are well marked in the distal two-thirds of the cusp. Inflammatory processes are usually characterized by varying degrees of deterioration of elastic tissue, and Hass (1939) has indicated that the chief deleterious factors are proliferation of fibroblasts, infiltration by inflammatory cells, accumulation of toxins and especially deposition of collagen. So far as I am aware, the phenomenon has not been described in heart-valves.

DISCUSSION

The present communication advances further evidence of the non-vascular nature of human heart-valves. The observations indicate also that in the subendothelium of the normal mitral valve there are several cell types, many of which under normal conditions are probably non-motile elements playing an important part in the general metabolism of the cusp. In valvulitis many are activated and produce other cell types. The reaction is primarily one of

local defence but it is evident also that it is secondarily associated with the establishment of new blood vessels within the cusp, if indeed it is not the actual stimulus for their formation.

The most probable explanation for the presence of blood vessels beyond their normal limit in valves is that they have been formed as a result of previous injury to the cusps. The exact nature of the injury is not understood in every case, but the recent work of Gross (1937) clearly indicates that rheumatic fever which had gone on to complete healing was responsible for the formation of blood vessels in 44 apparently normal atrio-ventricular valves. The present case, also of rheumatic origin, demonstrates the early formation of the vessels.

In the light of these observations, therefore, primary embolism can no longer be considered possible in the genesis of endocarditis in man.

On the experimental side much attention has recently been directed towards procedures involving the preparatory injection of substances, e.g. casein, starch, vaccines, etc., which are believed to effect a predisposition to the development of endocarditis. Dietrich (1926) and Thomson (1935) considered that these substances sensitized the valve tissue, and the explanation that such a state is a prerequisite for the development of endocarditis arose chiefly from the histological study of the valves in animals given intravenous injections of "preparatory substances" only. The principal changes noted were increase in the size and number of the cells, the appearance of histiocytes, and oedema of the valve tissue. Pfuhl (1929) has described pigmentation, vacuolation and destruction of histiocytes in the valves of animals treated with vaccines and casein, and the work of Semsroth & Koch (1930) indicated that there may be a disturbance of the detoxifying ability of the valve tissue in the genesis of endocarditis. These results are of some interest in the light of Silberberg's (1927) tissue-culture experiments which showed that macrophages containing lithium carmine are impeded in their phagocytic activity. The author (1938) has shown that the intravenous injection of "aleuronate" induced a marked increase in the number of cells in the subendothelium, and a proliferation of new blood vessels in the tricuspid valve of the rabbit, the vascular pattern of which conforms to the human type.

The results of animal experiments and the scanty data available for man indicate that in both a reticulo-endothelial function can be ascribed to the subendothelium. In man certain conditions, notably rheumatic fever, activate this zone and in animals the so-called "preparatory substances" have the same effect. If the exciting cause is continued, impairment of the reticulo-endothelial function and subsequent vascularization of the cusps result.

It must be pointed out, however, that the presence or absence of blood vessels in the normal cusps of experimental animals has not been sufficiently appreciated. Blood vessels occur normally in the heart valves of certain species and cannot be regarded as analogous to those sometimes found in man, which are of inflammatory origin. This obviously has a direct and important

bearing on the interpretation of experimental results which cannot, therefore, in every case be taken as strictly comparable with conditions observed in human valves. Thus the statement by Blahd *et al.* (1939) that a "preparatory substance" is unnecessary is possibly ill-founded, for the experimental animal which he employed, the dog, is one in which blood vessels can be demonstrated regularly in the normal valves. Clearly, in such a case primary attack from within the valve would be the most likely occurrence and doubtless accounted for the ease with which the authors produced endocarditis by the simple intravenous injection of haemolytic streptococci.

SUMMARY

Further evidence of the avascular nature of human heart-valves is advanced, and the histology of the anterior cusp of the mitral valve of a child aged 9 years is described. Early reaction of valve tissue and its relation to new formation of blood vessels is recorded in a case of rheumatic valvulitis in a child aged 8 years. The reticulo-endothelial function of the valvular sub-endothelium is indicated.

I wish to express my thanks to Prof. H. Turnbull for providing the pathological specimen and to Prof. J. D. Boyd for his interest in this work.

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THE EVOLUTIONARY SIGNIFICANCE OF THE EMBRYOLOGY OF THE AMPHIBIAN NEPHRIC SYSTEM

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THE ONTOGENY OF THE AMPHIBIAN NEPHRIC SYSTEM IN RELATION TO PHYLOGENY

MANY theories have been advanced to explain the arrangement of the vertebrate nephric system, and the most generally accepted is set forth by Goodrich (1930). He states that Craniata were provided originally with a paired series of segmental excretory tubules which coalesced to give rise to a longitudinal duct opening into the cloaca. This primitive archinephros then became specialized into pronephric and mesonephric regions. The pronephros showed a tendency to earlier differentiation, and the portion of the primitive archinephric duct formed in association with it tended to grow back independently to the cloaca so that species arose where it was completed before the formation of the mesonephric tubules, and the latter communicated with the already formed duct without contributing to its formation. Thus, for ontogeny to be in accordance with this phylogenetic view, it would be necessary for the pronephros to develop before the mesonephros and for the pronephric duct to grow caudally from the pronephric rudiment to the cloaca and be joined by the mesonephric tubules when they later developed. Such a plan of development has been described both in birds and in mammals, and in the chick this description has been confirmed by the experimental findings of Boyden (1924), Waddington (1937) and Grünwald (1937). However, in Amphibia the position has not been so clear. Early observations on the embryology of the nephric system in *Triton alpestris* by Mollier (1890) led him to the conclusion that the evidence at his disposal favoured the formation of the pronephric duct *in situ* and not as a caudal outgrowth of the pronephric rudiment. Field (1891), working on *Amblystoma punctatum*, was more definite in his opinion and concluded that the pronephric duct was derived from a proliferation of the somatopleure in each segment, his evidence being the similarity of the yolk granules in the two structures. Brauer (1902), however, investigated the rare Gymnophiona and here came to the conclusion that the pronephric duct was formed by an independent caudal growth of the pronephric rudiment. The position was thus established that with the exception of the Gymnophiona the pronephric duct in Amphibia was derived from a proliferation of the somatopleure *in situ*, a view expressed by the synonym "segmental duct". This position has been accepted by later writers and is repeated by Felix (1906) and Goodrich (1930). Burlend (1931) also accepts

this opinion on the mode of formation of the pronephric duct in Amphibia, and uses it as an argument against the current views on the phylogeny of the vertebrate nephric system.

Subsequent experimental work, however, is pertinent to the manner of formation of the pronephric duct. In Amphibia, several workers (Burns, 1934, 1936; Waddington, 1937; O'Connor, 1938) found that the formation of the pronephric duct could be prevented either by removing the pronephric rudiment or by placing a transplant at its caudal extremity and thus preventing any extension in a caudal direction. These results strongly suggest a formation of the pronephric duct from the pronephric rudiment but are not absolutely conclusive. There still remains the possibility that the pronephric duct is formed *in situ* in response to some stimulating force passed from the pronephric rudiment in a caudal direction. That is to say, the formation of the pronephric duct from the somatopleure in one segment may stimulate its formation in the segment immediately caudal and this stimulus may be removed by the above procedures. The formation of the entire pronephric duct from the pronephric rudiment is, however, established by vital staining experiments (O'Connor, 1938) where the pronephric rudiment was stained with Nile blue sulphate in the living embryo. When subsequent development was followed the dye could be traced into the pronephric duct in its whole length from pronephros to cloaca. These experiments were performed on *Amblystoma tigrinum* and *Triton taeniatus*, and in these species they establish the formation of the pronephric duct from the pronephric rudiment and not from the somatopleure of each individual segment, and they justify the generalization that this is the mode of formation of the pronephric duct in all amphibian species. To this extent, therefore, the embryology of the pronephric duct can be reconciled with accepted views on its phylogeny.

THE PURPOSE OF A VESTIGIAL PRONEPHROS

In higher vertebrates the pronephros appears first in the development of the nephric system, but soon degenerates and is never a functioning urinary organ. In Amphibia, however, it functions as an organ of urinary secretion for some time and in *Amblystoma punctatum* its bilateral removal results in the death of the embryo (Howland, 1921). Waddington (1937) investigated the importance of this vestigial organ in the chick and came to conclusions similar to those of Grünwald (1937). The results obtained by these two workers showed that the formation of the pronephric duct could be prevented in the caudal region of the embryo by making an incision in the path of the caudal extension of the duct. In such cases the mesonephros developed in the absence of the pronephric duct, and was much retarded in its development, tubule formation not taking place. Waddington therefore propounded the theory that the pronephros in the chick, although non-functional from the physiological point of view, gave rise to the pronephric duct which induced the formation of the mesonephros, and therefore the pronephros could be

regarded as having a morphogenetic function. Boyden (1924) has shown that the pronephric duct is of importance in the development of the avian cloaca. When the formation of the pronephric ducts is prevented, the middle chamber of the cloaca (urodaeal sinus) undergoes a deficient development. It would therefore appear that in the chick the pronephros, by giving rise to the pronephric duct, exerts an important influence on the development of both the mesonephros and the cloaca. It is now proposed to use existing evidence to test the applicability of this hypothesis to Amphibia where several species are available for consideration.

In order to establish the hypothesis in Amphibia it would be necessary to show:

(*a*) That the pronephros gives rise to the pronephric duct.

(*b*) That the pronephric duct stimulates the formation of the mesonephros.

(*c*) That the pronephric duct affects the development of the cloaca.

The origin of the amphibian pronephric duct. In *Amblystoma tigrinum* and *Triton taeniatus* the pronephric rudiment appears soon after the closure of the neural folds and consists of a thickening of the somatopleure at the ventrolateral border of the somites. It lies immediately beneath the ectoderm and so is visible as an external swelling and can be seen to lie in the third to seventh trunk segments. Experimentally it has been possible to place Nile blue sulphate in this pronephric rudiment so that localized portions of it were stained (O'Connor, 1938). The stain was applied to the embryos before the appearance of the rudiment by staining the cells that later would give rise to it. In one group of experiments the stain was placed in the third and fourth trunk segments. In this case it was found that the stain was confined to the portion of the rudiment found in these segments, and in no other portion of it. Subsequent observation as development proceeded showed that the dye remained confined to the pronephros, and at no stage was there any found in the pronephric duct. In a second series of experiments the dye was placed in the fifth to seventh body segments, and when the pronephric rudiment appeared the dye was confined to its caudal portion that formed in segments 5–7. Subsequently the dye in these cases extended caudally throughout the duct while at all stages the pronephros remained free from the dye. Therefore the pronephric rudiment can be divided into two portions:

(*a*) A portion derived from segments three and four, the cells of which can be traced into the pronephros but not into the pronephric duct.

(*b*) A portion derived from segments 5–7, the cells of which can be traced into the pronephric duct but not into the pronephros. Thus it is seen that the pronephros and the pronephric duct arise from different portions of the pronephric rudiment and these portions arise from different body segments. Hence, although the pronephric duct is derived from the pronephric rudiment it cannot be said to arise from the pronephros.

Moreover, it is possible to show that the formation of the pronephric duct is independent of any influence on the part of the pronephros. Experiments were performed where the portion of the pronephric rudiment giving rise to the pronephros was removed. The operation was performed before the pronephric rudiment had begun to extend caudally to form the duct and the tissue removed is indicated in Fig. 1. A flap of ectoderm was dissected back to expose the pronephric rudiment. The anterior portion of the rudiment was then removed, and the flap of ectoderm allowed to fall back into position. Embryos were sectioned at various periods after the operation with the results given below. The time periods are only approximate but the results apply to all three species employed, namely *Pleurodeles waltlii*, *Triton taeniatus* and a species of *Amblystoma*.

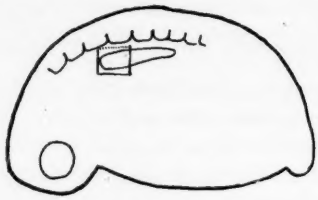

Fig. 1. Removal of the anterior portion of the pronephric rudiment.

(*a*) *One to ten days after operation.* On the operated side there was a normal formation of the pronephric duct. Lumen formation and union with the cloaca were similar to the normal side.

(*b*) *Ten to fifteen days after operation.* During this period there was some retardation of the development of the pronephric duct and in some cases there could be seen absence of the lumen and a varying degree of disorganization of the duct.

(*c*) *Later than fifteen days after operation.* In all such cases the integrity of the duct was restored in the region of the mesonephros and caudal to it, so that the pronephric duct on the operated side acted as the efferent duct of the mesonephros which underwent a normal development.

In the present discussion the results as seen in the first ten days after operation are of significance since they confirm the essentially different site of origin of the pronephros and of its duct and in addition show that the formation of the pronephric duct is not dependent on the presence of the pronephros. It is also worthy of note that in the absence of the pronephros the development of the mesonephros was in no way retarded (cf. Howland, 1926).

The effect of the pronephric duct on the mesonephros. The development of the Amphibian mesonephros in the absence of the pronephric duct has been investigated by several workers (see O'Connor, 1939). The results obtained

show considerable variation, and the following species may be quoted as indicating this variation. In *Pleurodeles waltlii* (O'Connor, 1939) in the absence of the pronephric duct there is no appearance of the mesonephros at all, in *Rana fusca* (Waddington, 1937) clumps of mesonephric cells appear but there is no formation of tubules, and in *Amblystoma maculatum* (Humphrey, 1928) there is an almost complete development of the mesonephros. The conclusion to be drawn therefore is that the influence exerted by the pronephric duct on the development of the amphibian mesonephros is extremely variable, and ranges from species where it is essential to development to species where it is unimportant.

The effect of the pronephric duct on the development of the amphibian cloaca. The normal development of the cloaca in the absence of one or both pronephric ducts has been recorded by O'Connor (1940) in *Pleurodeles waltlii, Triton taeniatus* and a species of *Amblystoma*. There is thus no evidence to suggest that the pronephric duct in Amphibia affects the development of the cloaca as Boyden was able to show in the case of the chick.

The position in Amphibia, therefore, is that the pronephric duct cannot be said to be derived from the pronephros, and although the duct stimulates the formation of the mesonephros in certain species there are however other species in which it has no such stimulating function. Finally in the Amphibian species investigated there is no evidence of any stimulating effect of the pronephric duct on the development of the cloaca. Thus it is not possible to give to the amphibian pronephros or pronephric duct the morphogenetic role ascribed to them in the chick. This leads to the possibility that as the urinary function of the pronephros is lost as a process of evolution this organ acquires a morphogenetic function. In Amphibia this change is perhaps suggested by the varying importance of the pronephric duct as a stimulator of mesonephric development. An interesting method of testing this suggestion would be to see whether in Amphibia there is any relationship between the importance of the pronephros as a urinary organ and its importance as a stimulator of the development of other organs.

On the other hand, if the findings in the chick are considered in the light of the findings in Amphibia it is possible to doubt the morphogenetic role ascribed to the pronephros and its duct. In the first place there is no evidence to establish the fact that in the chick the pronephros gives rise to the pronephric duct. A similar state of affairs to that which exists in Amphibia has not been excluded. In the second place the results in Amphibia give rise to difficulties in applying the results in the chick to other avian species, or to other orders of vertebrates, for the findings regarding the development of the amphibian mesonephros raise the possibility that a similar variation in the importance of the pronephric duct in the development of this organ may exist in other orders as well as in Amphibia. This cannot be excluded by investigations applied to one particular species.

SUMMARY

1. By experimental methods it has been shown that the pronephric duct in Amphibia is an independent caudal growth of the pronephric rudiment. The ontogeny of this duct is thus in accordance with accepted views on its phylogeny.

2. A morphogenetic role has been ascribed to the pronephros in the chick. This role cannot be established in Amphibia and the significance of this is discussed.

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NOTE ON THE INDEPENDENCE OF SESAMOID AND EPIPHYSIAL CENTRES OF OSSIFICATION

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Parsons (1904) suggested that many epiphyses have been derived phylogenetically from sesamoid bones. This conception he based primarily on the condition he found in lizards, where there is developed in the triceps tendon, before it reaches the olecranon, a separate cartilaginous or bony element, the ulnar patella. In mammals, with the exception of bats, there is no ulnar patella, and Parsons suggested that the bone had fused with the ulna and become its epiphysis. In *Sphenodon* he described the centre as separate in early life but as fused with the ulna in later development.

The opposite point of view, that a sesamoid could be derived from a separated part of a long bone, was put forward by Pearson & Davin (1921). A review of the histological structure of the bones however does not support either of these theories.

In typical lizards such as *Lacerta* (Fig. 1), the ulnar patella (*pat.*) is well developed as a bony nodule which articulates with the humerus and the ulna, but is completely separated from both bones by the articular cavity. In the young animal the upper end of the ulna shows a large secondary centre of calcified cartilage (*c.c.s.c.*) replaced in part of its extent by bone marrow (*b.m.*) and endochondral bone (*e.b.*). At the insertion of the triceps tendon another centre is developed by intratendinous ossification (*i.t.c.*), consisting of a thick mass of bone, and the cartilage in contact with it is calcified (*c.c.i.c.*). The two centres are separated by a sheet of hyaline cartilage. A similar but earlier stage is figured by Nauck (1936), while in an adult animal figured by Vialleton (1924) the two secondary centres appear to have joined to form a single centre still separated by the growth cartilage from the shaft. Other lizards agree in structure with *Lacerta*. In an *Ameiva* for instance the two centres were distinct and each had its own system of marrow cavities and endochondral bone. Thus in lizards the sesamoid, the intratendinous ossification at the insertion of the tendon, and the main epiphysial centre are separate entities.

In mammals the ulnar patella when present and the ulnar epiphysis have been developed along similar lines, and there is no reason to believe that they are interchangeable structures. Possibly the "double epiphysis" found occasionally at the upper end of the ulna in man (Fawcett, 1904) is constituted by the intratendinous and main epiphysial centres, but their internal structure is unknown.

In *Sphenodon* (Fig. 2) there is no ulnar patella, but the other two centres are distinct. In the stage figured the main epiphysial centre (*c.c.s.c.*) consists of a diffuse mass of calcified cartilage, as described elsewhere in other bones (Haines, 1939). The intratendinous ossification (*i.t.c.*) is well developed at the insertion of the triceps, but the cartilage in contact with it is still hyaline. In the adult animal the whole of the cartilage between the intratendinous bone

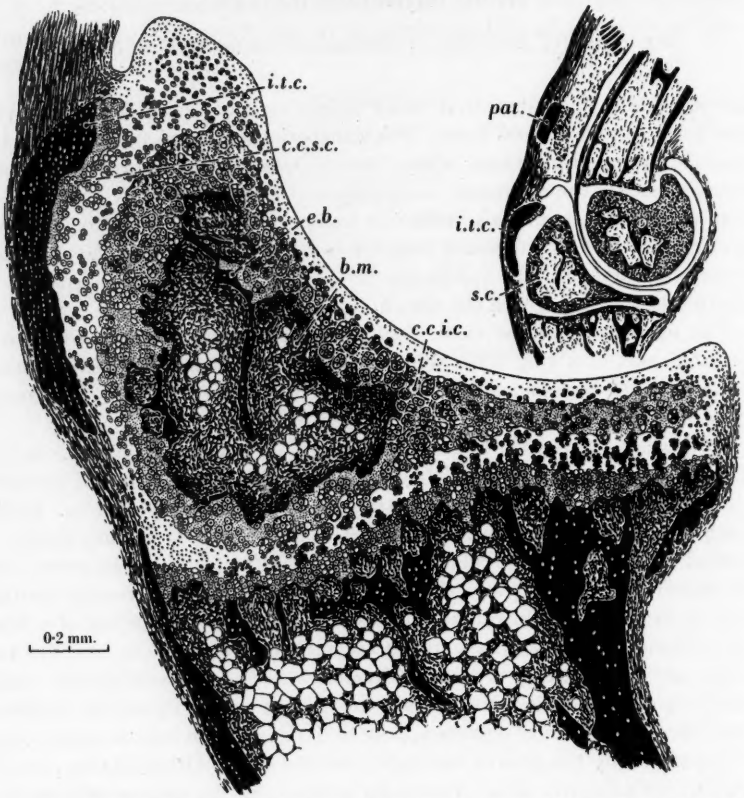

Fig. 1. Longitudinal section of the upper end of the ulna of a young *Lacerta*. Inset: elbow-joint on a smaller scale.

and the shaft is ossified. It is now clear that Parsons (1904), not having access to microscopic preparations, mistook the intratendinous bone for an ulna patella, while his observation that this bone later joined the shaft was of course accurate. But clearly *Sphenodon* does not illustrate any transition between a sesamoid and an epiphysial structure.

In the knee-joint of lizards the structure is similar to that in the elbow, with a patella, a main epiphysial centre for the tibia, and an intratendinous centre

at the insertion of the patellar tendon. In a young *Mabouia* (Fig. 3) the epiphysial centres were developed only as diffuse calcifications in the cartilage (*c.c.s.c.*), while the intratendinous part of the patella was bony (*pat.*), though the ossification had not yet spread into the deeper tendino-vesicular tissues (*t.v.t.*), and the intratendinous ossification (*i.t.c.*) was but a small nodule. In birds the intratendinous ossification is unknown, but the others are distinct, and it seems more probable that the patella and cnemial crest are grooved

Fig. 2. Longitudinal section of the upper end of the ulna of a young *Sphenodon*.

similarly by the muscles related to them than that they are, as Pearson & Davin (1921) suggest, separate parts of a single bony mass which owe their similarity of contour to a community of origin. In mammals the three centres are well developed, the centre for the tuberosity of the tibia having been found to develop by an extension of an intratendinous ossification by Bidder (1906) in the rabbit.

In primitive reptiles no secondary centres are found, at least up to the end of the Triassic period (Haines, 1938), and the patella is similarly unknown. Thus, since their phylogenetic lines had already separated at this time, the lizards, birds and mammals have developed the centres independently as neomorphs,

as suggested by Camp (1923) for the patella in lizards, not as an inheritance from a common stock, as suggested by Pearson & Davin (1921) for the patella of mammals and birds.

Finally, examination of the microscopic structure of the bones does not support the supposition of Moodie (1908) and others that certain epiphyses of

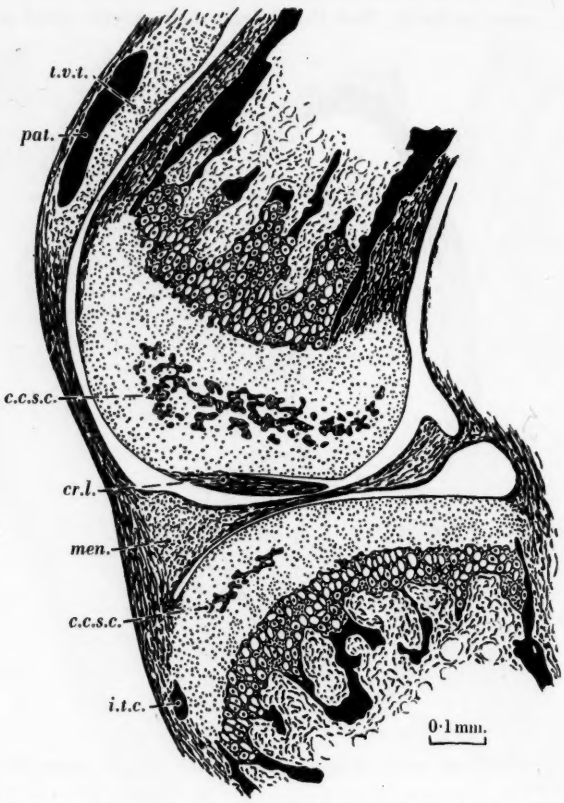

Fig. 3. Longitudinal section of the knee of a young *Mabouia homocephala*.

the metatarsal and metacarpal bones of lizards are derived from carpal elements fused on to the long bones. The structure of these bones as seen by Heidsieck (1928) and in my own preparations is normal in every way, and bears no resemblance to known examples of fusion between separate bones.

In general, histological studies suggest that these centres, once developed in the various phyla, retain their nature with remarkably little change.

KEY TO LETTERING

b.m. bone marrow; *c.c.i.c.* calcified cartilage associated with intratendinous centre; *c.c.s.c.* calcified cartilage of secondary centre; *cr.l.* cruciate ligament; *e.b.* endochondral bone; *i.t.c.* intratendinous centre; *men.* meniscus; *pat.* patella; *s.c.* secondary centre; *t.v.t.* tendino-vesicular tissue.

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A STUDY OF THE SUBCORTICAL CONNEXIONS OF THE OPTIC TRACT SYSTEM OF THE FERRET, WITH SPECIAL REFERENCE TO GONADAL ACTIVATION BY RETINAL STIMULATION

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INTRODUCTION

The occurrence of seasonal variations in both plants and animals is an observation as old as man, but not until comparatively modern times has any attempt been made to inquire into the precise mechanism of this rhythm. Throughout the nineteenth century it was believed that the important factor underlying this periodism was seasonal variation in temperature—a view derivative from Linnaeus, who published in 1793 what appears to have been the earliest investigation of the phenomenon (Rowan, quoting F. Smith, 1933). However, in 1920, the work of Garner and Allard showed conclusively that the important factor in the growth and maturation of various species of plants was rather the relative length of day to night. Five years later Rowan (1925) demonstrated that this was also true of birds; thus finches of the northern hemisphere, which normally only breed in spring, could be brought into full breeding condition in midwinter by daily subjection to gradually increasing periods of artificial illumination, additional to the normal hours of daylight. This response could only be attributed to retinal stimulation, since it was elicited in spite of exposure to intense cold (Canadian winter). Although this effect of visual stimulation was scientifically new, natural philosophy had long been aware of it, for it was apparently made use of empirically by both Dutch and Japanese bird-fanciers (Rowan, 1938*b*).

Research has now shown that gonadal activation follows retinal stimulation in other animal species; amongst mammals, the ferret has most commonly been used to demonstrate this. A brief historical review of the experimental work with the ferret may therefore be presented. Normally the female ferret is in the anoestrous phase between (approximately) September and the end of the following March. In 1932 Bissonette observed, however, that ferrets could be brought into full oestrus during this period by subjecting them daily—after the fall of darkness—to artificial illumination for 6–6½ hr. Male ferrets treated similarly showed well-developed accessory organs and readiness to copulate, but matings were sterile. Microscopical examination of the testes proved that, though the testicular interstitial tissue was fully developed, no spermatozoa, and only few spermatids, were present. Bissonette (1935) later found that normal spermatogenesis was induced in male ferrets, and fertile matings obtained, if short progressively increasing periods of illumination

were substituted for his earlier method. Hill & Parkes (1933) confirmed the sexual response of the ferret to experimentally increased retinal stimulation, and demonstrated that it was completely abolished by hypophysectomy. This latter result has been corroborated in birds by various authors; the place of the anterior pituitary as an intermediary in gonadal activation is well established.

Conversely, the effect of reducing visual stimulation was also tested experimentally. Thus Hill & Parkes (1934) kept five female ferrets in darkness for 23½ hr. daily from the end of January onwards; they reported that, in spite of this, four animals showed the beginning of vulval swelling (indicative of oestrus) well within the normal range of time. They commented that "it was noticeable, however, that the time taken for the vulva to become enlarged fully was longer than normal". In the fifth animal, the recurrence of oestrus was undoubtedly delayed, since no signs of gonadal activity were observed until 16 May. Marshall & Bowden (1934) also subjected female ferrets to "incomplete" darkness between the middle of April and the middle of July. Of seven animals, "four were beginning to come on heat before they were put into the dark room. The darkened conditions did not prevent them from coming fully on (heat)...the other three which were anoestrous at the commencement remained so throughout." Similarly, Bissonette (1936) reported that the reappearance of oestrous phenomena was markedly delayed in normal ferrets, if they were provided with light-tight hoods covering their eyes. Other experiments yielded comparable results. Marshall & Bowden described a ferret blinded in both eyes by cataract, which never came on heat at any time between December 1932 and July 1935; this finding was confirmed by Bissonette (1936). Bissonette (1936) also reported that section of the optic nerves delayed oestrus in female ferrets. Le Gros Clark *et al.* (1939) found that, after section of the optic nerves in four animals, two never showed any gonadal activity; of the remaining two ferrets, one came into full heat at the normal time, whilst the other showed delay, since only very slight vulval swelling was observable at the time of autopsy (10 May).

Thus the existence of a relationship between retinal stimulation and gonadal activation has been conclusively proved in the ferret by two methods of approach: (*a*) by augmentation, and (*b*) by either diminution or complete elimination, of retinal stimulation.

Retinal stimulation is therefore important to the animal in a manner which might perhaps be additional to, and different from, the ordinary visual perception of objects. On the assumption that gonadal activation might be a special response, it became a matter of considerable interest and importance to discover whether there is a special pathway from the retina concerned in its mediation. The work of Le Gros Clark *et al.* (1939) was undertaken to determine what the route of such a pathway might be. None of their experiments led to conclusive evidence for the solution of this problem, but the results did suggest the possibility that "the normal response of the pituitary

to retinal stimulation depends on impulses passing either to the ventral nucleus of the lateral geniculate body, or to the subthalamus by way of the accessory optic tracts". To this list may be added the so-called "dorsal hypothalamic root" of Frey.

The aim of the present study has therefore been to elucidate the anatomy of those pathways which might mediate the gonadal response of the ferret to retinal stimulation, and, in general, to provide a general survey of the anatomy of the optic tract system of this animal.

MATERIAL AND METHODS

The material made use of in the present investigation consisted of eighteen series of ferrets' brains; in addition there were available for comparison four series of rats' brains, and a single series of the rabbit. These preparations consisted of serial sections of the brain stem after removal of the cerebral hemisphere, extending from the optic chiasma rostrally to the inferior colliculus caudally. The plane of section was approximately parallel to the course of the optic tracts.

The rat and rabbit brains, as well as much of the ferret material used, were provided from earlier work of Prof. Le Gros Clark, who kindly placed them at my disposal.

The nature of the lesions and the staining techniques employed may most simply be presented in tabular form (Table 1). The nature of the lesion is indicated at the top of each column; the staining technique used in each case is shown in brackets after the animal's number.

Table 1

Normal control	Section of one optic nerve	Section of both optic nerves	Section of optic tracts	Midbrain lesion	Ablation of occipital lobes
F 85 (modified Ranson)	F 59 (Marchi) F 64 (Marchi) F 60 (Sereni & Young) F 61 (Sereni & Young) F 91 (modified Ranson) F 92 (modified Ranson) Rat 10 (modified Ranson) Rat 13 (modified Ranson) Rat 14 (modified Ranson) Rat 15 (modified Ranson) Rabbit 2 (Marchi)	F 25 (Bodian & Nissl)	F 16 (Weigert) F 39 (Bodian) F 20 (Loyez) F 22 (Loyez) F 50 (Loyez) F 71 (Loyez)	F 14 (Loyez) F 15 (Loyez)	F 23 (Nissl) F 93 (Marchi)*

* In F 93 only the right occipital lobe was removed.

The operative technique used in the ferret material has been described in detail elsewhere (Le Gros Clark *et al.* 1939), and no description of it is therefore necessary.

RESULTS

The optic chiasma

Special attention has been given in the present study to the form of the chiasma of the ferret, and to the disposition of fibres within it. It has been asserted (Frey, 1937) that, in the guinea-pig (Frey has also used human embryo material), there is a discrete "dorsal hypothalamic root" of the optic tract. According to Frey, this root arises from the dorsal part of the optic chiasma, and after a short course ends in the region of the supra-optic recess of the hypothalamus, caudal and dorsal to the chiasma. Some of the fibres in this root are said to decussate in the midline before passing into the hypothalamus; others, more laterally placed, to remain uncrossed. In addition to these fibre components of the hypothalamic optic root, Frey described a fine bundle of unmyelinated fibres, arising from the posterior margin of this hypothalamic root, which passes caudally on either side of the third ventricle to the median tuber nucleus of the hypothalamus.

In order to test the accuracy of Frey's observations, two Marchi series of ferrets' brains, after section of the left optic nerve, have been studied.

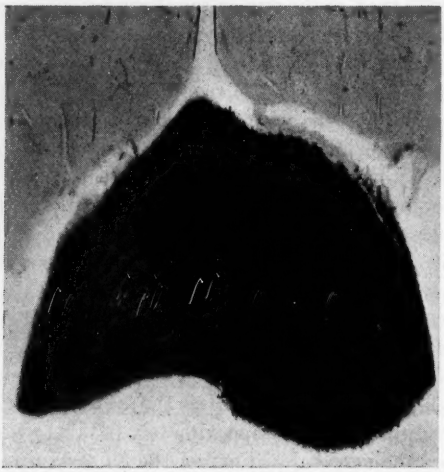
In vertical frontal sections the chiasma of the ferret shows a characteristic dorsal hump. Oblique frontal section alters the apparent shape of the chiasma; thus, in sections taken in a plane approximately parallel with the course of the optic tracts across the cerebral peduncles, the chiasma appears crescentic or biconvex, in either case with its maximum convexity dorsally. As the obliquity of section increases, its plane more and more approaches that of a tangent to the dorsal hump of the chiasma. Thus, in some slides of the ferret's brain, where this tangential plane of section has been most nearly achieved, the chiasma appears unconnected with the optic tracts. Study of more caudal sections establishes, of course, the continuity of the optic tracts with this seeming "median" root.

Disposition of the fibres in the chiasma

In the ferret Marchi preparations, after section of one optic nerve, it is found that the osmic granulation of the crossed optic tract is much greater than that of the uncrossed tract; the relative degree of degeneration in the two sides indicates that approximately two-thirds of the fibres arising in one retina cross in the chiasma, to reach the optic centres of the contralateral side; the remaining one-third are uncrossed.

In oblique frontal sections of the chiasma, fibres showing degeneration are seen running upwards towards the convex dorsal hump (Text-fig. 1). In Text-fig. 1 it is quite clear that the greater number of these fibres, having run upwards, then loop down into the optic tract of the opposite side. Since

this section is approximately parallel with the course of the optic tract, i.e. oblique to the axis of the brain stem, the U-shaped loop formed by decussating retinal fibres must have its convexity directed not only upwards but also backwards. Other fibres can be seen in Text-fig. 1 in the more dorsal part of the chiasma, which give the appearance of running directly towards the wall of the supra-optic recess. Study of serial sections shows that they do not end in this region, and that more caudally they pass down into the optic tract of



Text-fig. 1. F 64. Marchi. Magnification ×28. Frontal section through chiasma, after section of the left optic nerve. Degenerated fibres may be seen crossing in the chiasma. Dorsally, many fibres appear to run directly to the wall of the supra-optic recess.

the opposite side. The loop formed by these fibres, therefore, has its apex more caudally, as well as more dorsally, than that of the majority of fibres which have been described above.

In addition, in sections through the chiasma farther caudally than that illustrated in Text-fig. 1, fibres showing the Marchi reaction can be traced from the chiasma into the hypothalamus in close relation to the wall of the third ventricle (Text-fig. 2). Careful study, in Marchi sections, of these retinal fibres establishes the fact that they are really aberrant fibres of the optic decussation which eventually rejoin the crossed optic tract. For, in Marchi series after section of the left optic nerve, in sections in which they first appear, these fibres are limited to the hypothalamus to the left of the midline (Text-fig. 2). In more caudal sections, they are seen more or less symmetrically arranged on both sides of the midline. Still farther caudally, degenerated fibres are found in the ventral hypothalamic substance only to the right of the midline.

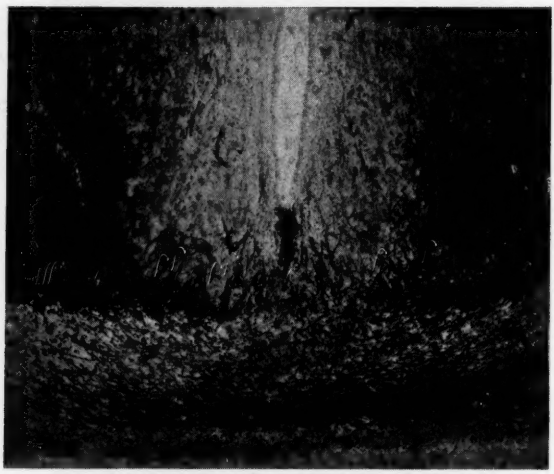
Since in this experiment only the left optic nerve was severed, degenerated fibres showing the Marchi reaction can only have had their origin in the left retina. It is clear therefore that these aberrant fibres do not really have a hypothalamic destination, but having made this detour rejoin the crossed optic tract.

Text-fig. 2. F 64. Marchi. Magnification × 111. Frontal section through chiasma at a more caudal level than that illustrated in Text-fig. 1. To the right of the third ventricle (*v.*), degenerated fibres leave the chiasma and pass dorsally into the substance of the hypothalamus. After an intrahypothalamic excursion these fibres rejoin the crossed optic tract. Pseudo-Marchi reaction along blood vessels is shown at *a*.

In a Weigert-Pal preparation of the ferret brain after section of both optic nerves, numerous short, undegenerated, myelinated fibres are seen running vertically in the ventral hypothalamus just dorsal to the posterior half of the chiasma (Text-fig. 3). These fibres, which run for short distances, are presumably short internuclear hypothalamic neurons; at all events, they can have no connexion with the retina. In normal material they might easily be mistaken for myelinated fibres of retinal origin which leave the chiasma; for this reason, they should be noted as a possible source of error in the interpretation of normal material.

Discussion. It is now possible to attempt some correlation of our findings with those of Frey.

It has been made clear that the chiasma of the ferret is characterized by a dorsal hump, and that more or less tangential section of this hump alters the apparent shape of the chiasma, so that it may even appear as an isolated median root. In the brain of the guinea-pig the dorsal hump of the chiasma is even larger than that of the ferret. It is obvious that the discrete median "dorsale hypothalamische Wurzel" of Frey (who used the guinea-pig) must be an illusion, produced by tangential section of the dorsal chiasmatic hump.



Text-fig. 3. F 16. Weigert-Pal. Magnification ×70. Frontal section through caudal part of chiasma in a ferret after section of both optic nerves; all retinal fibres have disappeared. Numerous short myelinated fibres are seen dorsal to the chiasma in the hypothalamic substance. In normal material these fibres might be mistaken for optic components. The commissure of v. Gudden is seen decussating in the ventral part of the degenerated chiasma.

This thesis is supported by the fact that the legends under the illustrations in Frey's paper, in which the "dorsal hypothalamic root" is shown, give the plane of sections as "horizontal". Such sections would undoubtedly be tangential to the dorsum of the chiasma.

The existence of a "hypothalamic optic root" as a discrete anatomical entity may therefore be dismissed. Frey described in his hypothalamic optic root myelinated fibres of retinal origin, which end in relation with nuclear masses of cells in the borders of the supra-optic recess, and with cells of the ependymal wall of the recess. These fibres are, according to Frey, partly crossed and partly uncrossed. In the ferret, it has not been possible to confirm this statement. An explanation of this observation of Frey may be provided by our findings in the ferret. It has been noted that those fibres seen in Text-fig. 1

in the dorsal part of the chiasma, which seem to run to the wall of the supra-optic recess, are found, more caudally, to pass down into the optic tract of the opposite side. It seems probable that Frey's claims for crossed and uncrossed fibres, which end in relation to cells in the neighbourhood of the wall of the supra-optic recess, are based upon a false conception of the course of fibres similar to those which we have described in the ferret. Frey also states that from the posterior border of his "dorsal hypothalamic root", fibres pass caudalwards as fine bundles on either side of the third ventricle towards the median tuber nuclei. These fibres are particularly well illustrated in Figs. 37 and 38 of his paper. It is possible that these fibres are homologous with the retinal fibres in the ferret which, in decussating, pursue an aberrant intra-hypothalamic course. Against this view, however, are two facts: first, these fibres are unmyelinated, and secondly, the plane of the section is horizontal and the fibres appear to run, without any inclination dorsalwards, directly caudally for some distance—so far, in fact, that they could not possibly rejoin the optic chiasma. On the other hand, it is extremely probable that these fibres do not belong to the optic system at all; possibly they are analogous with those myelinated fibres seen in the ferret, dorsal to the chiasma, after section of both optic nerves, when of course all fibres with retinal connexions have degenerated.

The supra-optic commissure systems

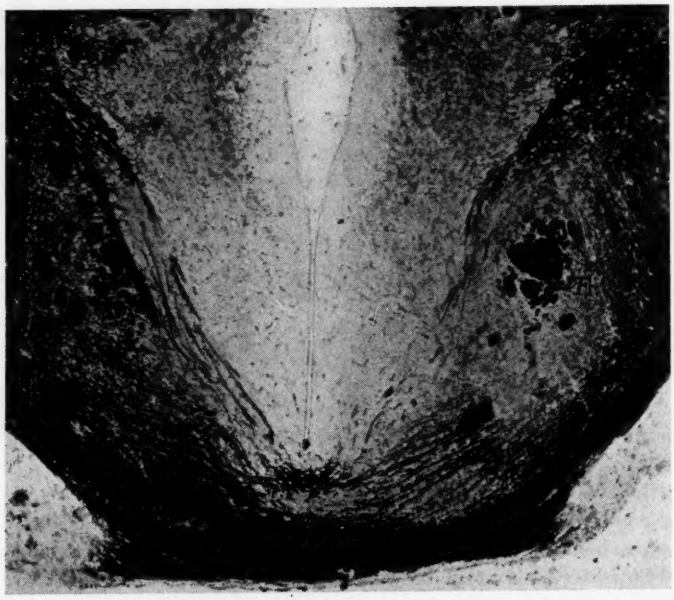
Three commissural systems are included under this head; they are the commissures of Ganser, Meynert and van Gudden, respectively. Their importance lies in the very intimate relationship they bear to the chiasma, and also to the anterior accessory optic tract of Bochenek.

These systems have been studied in the ferret brain, using material which had been stained by the Weigert-Pal method after section of both optic nerves. Several Loyez series, after bilateral section of the optic tracts, were also studied; but, since the results were substantially the same as in the Weigert material, detailed reference will be made only to the latter series.

The commissure of Ganser (Text-fig. 4). This commissure lies below the floor of the third ventricle and above the optic chiasma. Fibres belonging to the system show clearly in Weigert sections as thick myelinated fibres which do not run in compact bundles. From their decussation below the floor of the third ventricle they can be traced laterally and dorsally; chiefly they pass medial to the columna fornicis to reach the region of the subthalamus. Beyond this point they cannot be followed. Some fibres of this commissure, however, take a different course, running inferior to the columna fornicis to enter the medial forebrain bundle. There are indications that they pass through the medial forebrain bundle to join the system of fibres traversing the lateral surface of the cerebral peduncle.

The exact connexions of Ganser's commissure in our ferret material cannot be clearly defined; it probably constitutes a link between the subthalamic

regions of opposite sides. This is in agreement with the suggested role of this commissure in the opossum (Tsai, 1925). It may be mentioned that Gurdjian (1927) traced fibres of Ganser's commissure in the rat into the medial forebrain bundle, and, through it, into the subthalamic area. It was not possible, in the ferret brains available, to verify this. Gurdjian believed the function of this commissure to be in connecting the subthalami of opposite sides. In addition, he noted, in Cox-Golgi preparations of the rat brain, that a scattered group of



Text-fig. 4. F 16. Weigert-Pal. Magnification ×30. Frontal section through the brain stem just caudal to the chiasma. The thick myelinated fibres of Ganser's commissure are clearly shown, passing dorso-medially to the columna fornicis (*F*), after decussating in the floor of the third ventricle. The commissure of Meynert is seen more ventrally. The degenerated optic tracts lie ventro-laterally on either side of the brain stem.

cells lying in the ventral hypothalamus, through which fibres of Ganser's commissure pass, contribute axons to the commissure. In the rat at least, this system may therefore play some part in connecting the hypothalamus with the subthalamus.

The commissure of Meynert (Text-fig. 4). Meynert's commissure lies slightly caudal and dorsal to the optic chiasma, and ventral to the commissure of Ganser. It consists of finer myelinated fibres than the latter; the fibres composing this system in the ferret are very numerous and closely packed. From its decussation it may be traced laterally and dorsally, lying to the

medial side of the optic tract. It penetrates the medial forebrain bundle, in which many of its fibres appear to bend dorsally and medially to come into relation with the region of the subthalamic nucleus. Other fibres, however, continue over the ventral surface of the cerebral peduncle, medial to the optic tract. At least some of these fibres appear to pass through the septa of the cerebral peduncle to reach the zona incerta. In addition to the fibres of this commissure which pass to the subthalamus, Gurdjian traced (in the rat brain) fibres which join with the cortico-tectal fibres to run to the tectum, and other fibres which are intimately connected with the ventral nucleus of the lateral geniculate body. These findings may apply also to the ferret; in the Weigert series after section of both optic nerves, many normal fibres running in the so-called "optic tract" can be seen, but they clearly have no retinal connexions at all. It is difficult to assign these fibres to one commissural system rather than another; they must belong to the commissures of Meynert and Gudden, but it is impossible to make out their exact connexions or destinations.

Tsai has stressed the close relation of the anterior accessory optic tract and the commissure of Meynert. Observations on Marchi ferret preparations after section of one optic nerve, demonstrate that fibres of retinal origin showing the Marchi reaction are seen in the position occupied by the commissure of Meynert (as identified in Weigert and Loyez series of the ferret's brain). Whether these degenerated fibres are, in fact, the fascicles of the anterior accessory optic tract or not will be discussed below. In any case, the classical description of the anterior accessory optic tract shows that it must be very closely related topographically to the commissure of Meynert.

The commissure of Gudden (Text-fig. 3). In a Weigert series after section of both optic nerves, the fibres of the commissure of Gudden may be seen decussating in the ventral and posterior part of the chiasma. Posteriorly, it is impossible to distinguish the commissure of Gudden from the anterior portion of Meynert's commissure. From its decussation, the fibres of Gudden's commissure run dorso-laterally in the posterior part of the optic tract, apparently directly to its lateral side. It has been mentioned above that, even after bilateral section of the optic nerves resulting in degeneration of the optic tracts proper, so many undegenerated fibres remain that the picture presented is confusing. The connexions of Gudden's commissure in the ferret cannot be distinguished in the material available. Gudden's commissure is generally believed to connect the medial geniculate bodies of opposite sides, and perhaps also the inferior colliculi.

The anterior accessory optic tract of Bochenek

This small fascicle is generally described as arising from the optic tract just distal to the chiasma; it then runs dorso-laterally on the medial side of the optic tract in close relation with the commissure of Meynert. As it crosses the lateral surface of the cerebral peduncle, it turns dorso-medially and

penetrates the peduncle to reach the corpus Luysii (subthalamic nucleus). A number of fibres of this tract may also pass over the dorsal surface of the peduncle to reach the corpus Luysii. This tract was first stated to be derived from the main optic tract by Stilling in 1878. Its existence as an optic pathway was not, however, generally accepted till the work of Bochenek (1908) thirty years later. Bochenek made use of the Marchi technique, after removal of one eye, in several mammalian species. This work was confirmed by Pavlov (1900) and Loepp (1912). Both these workers used the rabbit for their experiments. Loepp gave the name "radix Luysii" to this optic component.

There is no evidence for the existence of this tract in the ferret. In two Marchi series, after section of the left optic nerve, no degeneration could be found which corresponds in its relations to the anterior accessory optic tract. Immediately distal to the chiasma a few fibres, showing the Marchi reaction, can be observed running in the ventral hypothalamus, medial to the main optic tract. They occupy a position amongst the fibres of Meynert's commissure (as identified in Weigert and Loyez series). If these degenerated fibres are followed in serial sections, they are found, rostrally, to arise from the chiasma, and caudally, to rejoin the main optic tract ventral to the cerebral peduncle. There is no degeneration which can be followed in serial sections either through the peduncle to the subthalamic nucleus or dorsally over the peduncle and thus down to the nucleus. Some slight pseudo-Marchi granulation is scattered irrelevantly through the peduncle, and also along blood vessels penetrating the latter, but this appears to be without significance.

From a review of the literature, it seems that the evidence for the existence of the anterior accessory optic tract is more or less confined to the rabbit and the rat. Thus, in the rabbit, it has been described by Pavlov (1900), Bochenek (1908) and Loepp (1912). Amongst our own material was a single Marchi series of a rabbit's brain after enucleation of the left eye. This series extends from the middle of the chiasma anteriorly, to about the middle of the superior colliculus posteriorly. Approximately three-quarters of the fibres from one retina cross in the chiasma, the remainder being uncrossed. There is a considerable amount of pseudo-Marchi reaction scattered in the internal capsule, the cerebral peduncle, the region of the subthalamus and the ventral hypothalamus. This appearance is found on both sides, though more markedly on the right (i.e. contralateral) side. The slides are therefore of little value, except for two points: (i) There is no sign of fibres penetrating the peduncle to reach the subthalamic nucleus, the whole picture being entirely unlike that figured by Loepp (1912). (ii) On the dorsum of the peduncle, many fibres bend medially, and run as if about to circle downwards on the medial aspect of the peduncle. Such a course for fibres of the anterior accessory optic tract has been described (see above). In our rabbit material, however, the fibres which at first give this impression are found, when followed caudally in serial sections, always to bend upwards again to run through the ventral nucleus of the lateral geniculate body. The sigmoid course of these fibres may have misled earlier workers. This material, however, does not provide sufficient evidence to dis-

prove the existence of the anterior accessory tract, which has been recognized in the rabbit by many other workers.

After enucleation of one eye in the rat, Kosaka & Hiraiwa (1915; quoted by Lashley, 1934) were unable to trace the anterior accessory tract into the cerebral peduncle; they regarded it as an aberrant bundle of optic fibres, which later rejoined the main optic tract. Le Gros Clark (1931) stated that the anterior accessory optic tract was clearly demonstrable in the rat in Marchi preparations; he was able, however, to follow it only as far as the ventral surface of the peduncle; no degenerated fibres were seen penetrating the peduncle.

The findings of these authors in the rat, therefore, seem to agree closely with our observations in the ferret, i.e. that the so-called anterior accessory tract is composed of aberrant fibres, which leave the tract just behind the chiasma, run amongst the fibres of commissure of Meynert and later rejoin the main optic tract.

On the other hand, Lashley (1934) and Chang (1936) have identified this tract in Marchi preparations of the rat's brain after unilateral enucleation; they found Marchi degeneration only in the crossed accessory tract. According to Lashley, the course of the anterior accessory tract was as follows. The fibres, having left the chiasma, travel at first in the substance of the hypothalamus with the commissure of Meynert as far as the inferior margin of the peduncle. At this point the tract divides into superior and inferior branches. The superior branch rejoins the main optic tract, and a few of its fibres may be traced between the fasciculi of the peduncle, but they cannot be followed into the corpus Luysii. The inferior branch, which contains the greater number of fibres, joins the posterior accessory optic tract, and terminates with it in the nucleus opticus tegmenti. It is interesting to note that Overbosch (1927; quoted by Bodian, 1937) found in the rabbit that retinal lesions, which caused degeneration to appear in the anterior accessory tract, always caused degeneration also in the posterior accessory tract, and conversely. He suggested that the anterior accessory optic tract might be merely an aberrant part of the posterior tract. Lashley's observation in the rat seems to lend support to this view. It seems, therefore, that the existence of the anterior accessory optic tract in the rat is equivocal.

After intensive search of Marchi sections of the opossum's brain, Bodian (1937) failed to discover any degenerated fibres which might be called the anterior accessory optic tract. Bodian also studied the normal Weigert material of the opossum, which had been used by Tsai (1925); he was not convinced that the fibres labelled in Tsai's figures as the anterior accessory optic tract were, in point of fact, of retinal origin.

Barris *et al.* (1935) found no sign of the tract of Bochenek in Marchi series of the cat's brain after either unilateral or bilateral section of the optic nerves. They quote several other observers who had not been able to see this tract in the cat's brain (Probst, 1900; Kosaka & Hiraiwa, 1915; Brouwer *et al.* 1925). Barris *et al.* are of the opinion that the anterior accessory optic tract does not exist in higher mammals.

To summarize the position: the anterior accessory optic tract does not exist in the opossum, ferret or cat. It must be believed to exist in the rabbit. The existence of the tract in the rat is more questionable. It seems legitimate to suppose that findings in the rat correspond largely with our observations in the ferret. That is, the fibres seen in the rat do not agree with the classical description of the orientation of the anterior accessory tract, because they cannot be followed to the corpus Luysii through the peduncle (but cp. Chang, 1936, who claimed that fibres showing Marchi degeneration do reach this nucleus in the rat). The presumption is, therefore, that the fibres described under the heading of the anterior accessory optic tract by workers with the rat are homologous with those which we have described in the ferret as aberrant fascicles of the main optic tract.

It is also possible that considerable individual variation occurs, even in animals in which this tract has been shown conclusively to exist.

The posterior accessory optic tract

Typically the posterior accessory optic tract is described as a small bundle of fibres arising from the main optic tract just proximal to the lateral geniculate body. This accessory fascicle then passes caudally and ventrally over the cerebral peduncle to the interpeduncular space. Just anterior to the superficial origin of the oculomotor nerve it enters, and terminates in, the nucleus opticus tegmenti; this nucleus lies between the mammillary body medially and the cerebral peduncle laterally.

The tract was first recognized as part of the optic system by van Gudden (1870), who observed degeneration in the tract after removal of the opposite eye. Gudden gave the name of "tractus peduncularis transversus" to this bundle of fibres of retinal origin. The existence of this accessory optic path was confirmed by the experimental work of Pavlov (1900) and Loepp (1912) in the rabbit. These authors traced Marchi degeneration in the crossed tract after unilateral enucleation. Bochenek (1908) also found Marchi degeneration in this tract after similar procedures in a variety of mammals; he named the tract "fasciculus accessorius opticus posterior".

In the ferret brain a small and circumscribed bundle of fibres can usually be seen crossing obliquely over the lateral surface of the cerebral peduncle, occupying the topographical position of the tractus peduncularis transversus of other mammals. Attention was drawn to this by Le Gros Clark *et al.* (1939), and it was assumed that, as is the case in rodents, it represents a posterior accessory optic tract; but a further study of sixteen ferret brains makes it doubtful if this is the case, for the fascicle appears macroscopically not to rise from the main optic tract, but rather to be split off the brachium of the inferior colliculus. If this is so, it clearly cannot be a posterior accessory optic tract.

In Marchi sections of the ferret's brain, after section of one optic nerve, no fibres are seen which follow the course described as that of the posterior accessory optic tract, nor is any Marchi granulation seen either in the contra-

lateral or the ipsilateral nucleus opticus tegmenti. This experimental evidence shows therefore that, in the ferret, the posterior accessory optic tract probably does not exist (at least so far as myelinated fibres are concerned).

Recent work with the rat, the guinea-pig, and the opossum has, however, established the presence of this tract in these animals. Thus, in Marchi preparations of the rat's brain, degeneration in the tract has been observed after removal of the opposite eye (Le Gros Clark, 1931; Lashley, 1934; Chang, 1936). Tsang (1937) has noted atrophy of the tract in rat Weigert material after unilateral and bilateral enucleation. Frey (1937) has identified this bundle in Marchi preparations of the guinea-pig after removal of one eye; this confirms the earlier observations of Wallenberg (1904) and Castaldi (1923) in the same animal. Frey has given this tract in the guinea-pig the name of "basale optische Wurzel" (basal optic root), on the presumed homology (Marburg, 1903) of the posterior accessory optic tract and its nucleus with the basal optic root and ganglion ectomammillare of reptiles and birds.

In the opossum, Tsai (1925) described the course of the posterior accessory optic tract as seen in Weigert sections of the normal brain. Bodian (1937) found Marchi degeneration following the path described by Tsai as that of the posterior accessory tract, after enucleation of the opposite eye in the opossum.

Barris *et al.* (1935) were unable to find the posterior accessory optic tract in Marchi series of cats' brains after either one or both eyes had been removed. They conclude, from a review of the literature, that the evidence for its existence seems to be limited to the rodents and lower mammals. The absence of the posterior accessory optic tract in the ferret is in agreement with this thesis. Furthermore, if the anterior accessory optic tract is merely an aberrant portion of the posterior tract (see above: Overbosch, 1927), the absence of the anterior accessory optic tract in the ferret would not be surprising.

The lateral geniculate body

Anatomy of the ventral nucleus of the lateral geniculate body. In two Marchi series of the ferret's brain after removal of the left eye, degeneration is present in the ventral nucleus of the lateral geniculate of both sides; it is greater in the contralateral than in the ipsilateral nucleus. The Marchi reaction however appears very largely to be limited to bundles of fibres of retinal origin which run through the ventral nucleus on their way to the dorsal nucleus of the lateral geniculate body. Marchi granulation outside these fibres in the substance of the ventral nucleus is very slight: the appearance is therefore totally unlike that of the dorsal nucleus (see below), and is consistent with the view that this granulation seen in the ventral nucleus is related only to fibres of passage. The possibility remains however that unmyelinated fibres or the unmyelinated collaterals of myelinated optic fibres of passage of course may end in this nucleus. Our ferret material does not provide positive evidence on this point.

Cajal (1911) and Kappers (1921) both believed that collaterals of optic tract fibres ended in the ventral nucleus; Pavlov (1900), Loepp (1912) and Brouwer (1923), from study of Marchi preparations after unilateral enucleation in the rabbit, stated that fibres of the optic tract ended in the ventral nucleus of the lateral geniculate. In Marchi sections of one rabbit's brain which was at our disposal, the appearance of the degeneration is very similar to that described above for the ferret; certainly no positive evidence is presented that optic fibres end in the ventral nucleus.

Amongst recent workers the view that the optic tract makes no connexions either by direct fibres or by collaterals with the ventral nucleus of the lateral geniculate is widely held (Gurdjian, 1927; Lashley, 1934 (rat); Tsai, 1925; Bodian, 1937 (opossum); Barris *et al.* 1935 (cat)). The work of Tsang (1937) in the rat is also in support of this view. He observed that after either unilateral or bilateral extirpation of the eyes there is never atrophy of the cells of the ventral nucleus of the lateral geniculate, however long the period of degeneration. On the other hand after similar operative procedures the cells of the dorsal nucleus become shrunken, rounded and embryonic in form, with fewer processes than normal. Tsang believes this to be an example of atrophy from disuse. In the rat, therefore, it seems that there can be no close relation between the optic tract and the ventral nucleus of the lateral geniculate, either by myelinated or by unmyelinated fibres; for if there were, an atrophy of the ventral nucleus comparable with that of the dorsal nucleus might perhaps be expected.

In a Weigert preparation of the ferret's brain after section of both optic nerves, with the consequent complete degeneration of the optic tracts, numerous myelinated fibres are still present in the ventral nucleus of the lateral geniculate (see Pl. 1, fig. 1), which in normal preparations may easily be mistaken for component fibres of the optic tract. Many of these fibres appear to pass straight through the ventral nucleus and over the lateral aspect of the dorsal nucleus towards the tectum. The picture presented is somewhat confusing. These fibres probably belong chiefly to the commissures of Meynert and v. Gudden. Fibres have however been described running from the ventral nucleus of the geniculate to the tectum; e.g. Barris *et al.* (1935) observed a "tractus geniculatus ascendens" in the cat, which passes up dorso-laterally over the dorsal nucleus of the lateral geniculate. They suggested that this tract consists of fibres going only in one direction, i.e. towards the tectum. Rioch (1929) described geniculo-tectal fibres in the dog which arise in the ventral nucleus, and join with the retino-tectal fibres to pass superficially over the dorsal nucleus of the lateral geniculate to the midbrain. Le Gros Clark also has described geniculo-tectal fibres in the rat (1931). It is therefore probable that in the ferret a number of the fibres, seen in the ventral nucleus of the lateral geniculate and also passing over the superficial aspect of the dorsal nucleus, belong to this geniculo-tectal tract; it is however impossible in our Weigert material to make out this tract as a discrete bundle, distinct from the other

fibres. In Weigert preparations of the ferret's brain, many finely myelinated fibres are seen running from the ventral nucleus ventro-medially to the zona incerta. These constitute the geniculo-incertal tract which has been observed by many authors (Gurdjian (1927) in the rat; Barris *et al.* (1935) in the cat—these latter authors gave the name of "tractus geniculatus descendens" to the geniculo-incertal connexions; Rioch (1929) in the dog; Le Gros Clark (1930) in *Tarsius*). The incertal connexions of the corpus griseum pregeniculatum—the primate homologue of the ventral nucleus—have also been described in man and other primates (Balado & Franke, 1937).

Study of a Nissl preparation of the ferret's brain after bilateral ablation of the occipital lobes demonstrates that, whereas the cells of the dorsal nucleus undergo complete degeneration, the cells of the ventral nucleus remain apparently unchanged. The ventral nucleus of the lateral geniculate therefore has no connexions with the striate cortex.

Briefly, it is clear that the ventral nucleus of the lateral geniculate body of the ferret is connected with the zona incerta and probably also with the tectum; it has no connexions with the cortex. Its connexions with the optic tract have not been demonstrable in the material available; it seems legitimate to assume that they do not exist.

Anatomy of the dorsal nucleus of the lateral geniculate body. It was suggested towards the end of last century, on the basis of clinical and pathological studies, that there is a point-for-point representation of the retina on the striate cortex (Henschen, 1893; Wilbrand, 1890). If such a relationship between the retina and the visual cortex obtains, it is clear that "segregation" of the fibres from the various parts of the retina must exist throughout the visual system. Thus if there is in fact a complete cortical representation of the retina, it is to be expected that the dorsal nucleus of the lateral geniculate will also show a "projection" of retinal points, in so far as this nucleus functions as a relay centre for optic impulses to the cortex. From a study of human clinical material, Henschen concluded that the dorsal quadrants of the retina were projected dorsally, and the ventral quadrants ventrally, in the dorsal nucleus of the lateral geniculate. It has been shown however by more recent work that this view is incorrect, though representation of the retina in the dorsal nucleus is indeed present. Such a relationship between the retina and the dorsal nucleus has now been found to exist in a number of mammals, e.g. opossum, rabbit, rat, and cat, as well as in primates including man; representation most closely approximates to a point-for-point projection in primates. It is probable therefore that in the ferret localization of retinal points exists in the lateral geniculate. Such results as we have obtained support this hypothesis, and may be compared with the more detailed observations of other workers.

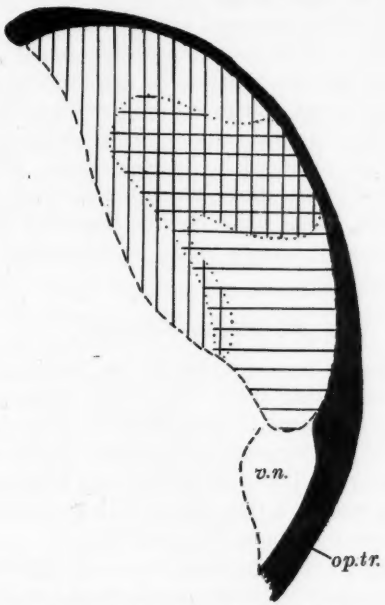
Study of two Marchi preparations of ferrets' brains after section of one optic nerve shows that there is much Marchi granulation present as a fine "dust" in the dorsal nucleus of both crossed and uncrossed sides. These granules are

scattered through the substance of the nucleus, and for the most part are not arranged in rows as if along some definitive pathway: i.e. the granulation appears to represent the terminal portions of myelinated optic fibres ending in relation to cells of the dorsal nucleus, and does not give the impression of being limited merely to the course of bundles of fibres of passage. This is in contradistinction to the picture presented by the ventral nucleus after similar operative procedures. The Marchi reaction in the crossed dorsal nucleus is seen to be both more dense and more widely distributed than in the uncrossed nucleus. Further, the distribution of degeneration is not identical on the two sides. In the uncrossed dorsal nucleus the distribution is as follows: in frontal sections through the rostral end of the dorsal nucleus, the greatest diameter of the nucleus is approximately horizontal, and in this portion of the nucleus the Marchi degeneration is seen to be limited to the medial one-third. Slightly more caudally the greatest diameter shifts so that it becomes more or less vertical. Granulation is now seen in the dorsal pole and upper two-thirds of the medial part of the nucleus. Further caudally, degeneration is limited to a horseshoe-shaped cap in the dorsal part of the nucleus; the medial limb of the horseshoe reaches considerably more ventrally than the lateral limb (see Pl. 2, fig. 4). In the caudal end of the nucleus the area of Marchi granulation is confined to a comparatively small oval, situated at the middle of the medial portion of the nucleus. At levels through the crossed nucleus corresponding to those described in the uncrossed nucleus, the areas of Marchi granulation are as follows: anteriorly where the longest diameter is horizontal, degeneration occupies the lateral three-quarters. When the long axis has shifted to become vertical, degeneration is seen throughout the nucleus except in its dorsal pole and medial one-third. Farther posteriorly, degeneration is again present throughout the nucleus, except for a narrow horseshoe-shaped band which has its apex dorsally and the medial limb of which extends much farther ventrally than the lateral (see Pl. 2, fig. 3). At the caudal end of the nucleus, Marchi granulation is limited to the ventral half of the nucleus. In the most caudal part of the crossed nucleus, Marchi degeneration is seen arranged in rows parallel with the greatest diameter of the nucleus; this appearance indicates degenerated fibres of passage belonging to the mesencephalic root of the optic tract which run through the dorsal nucleus to join the brachium of the superior colliculus.

To summarize these observations: it is seen that retinal fibres end everywhere in the crossed nucleus except in its dorsal pole and its most medial part. Conversely retinal fibres end in the dorsal pole and medial portion of the uncrossed nucleus. This segregation in the lateral geniculate of crossed and uncrossed fibres is not complete, so that a partial overlap occurs; this can readily be seen in Text-fig. 5; in this figure it is clear that the area occupied by Marchi granulation in the uncrossed dorsal nucleus is considerably larger than the area which shows no granulation in the crossed dorsal nucleus of the lateral geniculate body.

The projection of crossed and uncrossed optic fibres in the dorsal nucleus of the ferret thus appears comparable with the projection described in other mammals, e.g. opossum, rabbit, rat and cat (Minkowski, 1920; Brouwer, 1923; Brouwer & Zeeman, 1926; Putnam & Putnam, 1926; Overbosch, 1927; Lashley, 1934; Chang, 1936; Bodian, 1937).

In addition, in the above-mentioned animals, the localization of the retinal quadrants in the dorsal nucleus of the lateral geniculate has been worked out.



Text-fig. 5. Diagram illustrating the overlapping projection of the crossed and uncrossed retinal fields in the dorsal nucleus of the lateral geniculate body (prepared from photomicrographs in Pl. 2). The crossed projection field is represented by horizontal lines, and the uncrossed by vertical lines. *op.tr.* optic tract; *v.n.* ventral nucleus of the lateral geniculate body.

It is found that the ventral half of the contralateral retina is projected dorsally—the nasal quadrant being lateral and the temporal quadrant medial; similarly the dorsal half of the retina is projected ventrally—the nasal quadrant again being lateral and the temporal quadrant medial. The uncrossed portions of the temporal quadrants are projected most medially in the dorsal nucleus, partially overlapping the crossed temporal fields; the ventral half of the uncrossed temporal retina is projected dorsally in this area, and the dorsal half ventrally.

It seems probable that research on the projection of the retinal quadrants on the dorsal nucleus of the ferret will reveal similar relations.

Some correlation might exist between the cytoarchitectural structure of the dorsal nucleus of the ferret lateral geniculate and those areas which receive

respectively crossed and uncrossed optic fibres. A study of a Nissl preparation of the ferret's brain was therefore made: the dorsal nucleus shows some lamination, but its pattern is complex and cannot be readily analysed. It is impossible to integrate it with the localization of crossed and uncrossed retinal fibres as worked out in Marchi sections after unilateral enucleation. Two reasons for this failure may be offered. First, the findings in the ferret as to the representation of the retina in the dorsal nucleus are only of a gross nature, i.e. they are limited to observations on the projection of the *whole* of the crossed, and the *whole* of the uncrossed, retinal fields. Perhaps a more detailed analysis of the projection of the retinal quadrants might reveal a correlation. Secondly, Rioch (1929) has described in the dog the laminar structure of the dorsal nucleus: he states that though in frontal sections the picture presented is confusing, paramedian sagittal sections clearly demonstrate that there are four laminae which are arranged in sigmoid curves. Possibly parasagittal sections of ferret Nissl preparations would demonstrate a clear and simple lamination of this kind, which might then be correlated with observations on the projection of retinal quadrants in the dorsal nucleus. Further research along these lines is indicated.

The superior colliculus

The superior colliculus has been shown in a variety of mammals to have a laminated structure (Cajal, 1911; Tsai, 1925; Tsang, 1937; and others). This lamination is also present in the colliculus of the ferret, being clearly demonstrable in Nissl and Weigert preparations. In the enumeration of the layers, the nomenclature of Tsang (1937) has been followed:

Layer I: Stratum zonale: a thin superficial layer consisting of small marginal cells, and a number of fine myelinated fibres.

Layer II: Stratum griseum superficiale: a much thicker layer, which consists of comparatively scattered cells mostly rather smaller than those of the deeper layers.

Layer III: Stratum opticum: in this layer run the fibres of the mesencephalic root of the optic tract, and a deeper fibre component which arises in the cerebral cortex. This layer also contains medium-sized cells.

Layer IV: Stratum lemnisci: the fibres composing this layer belong to the lemniscal systems and to the brachium of the superior colliculus. The cells found in this layer are small and scattered, though a few are giant cells.

Layer V: Stratum album profundum: this layer contains small scattered cells and the efferent fibres of cells in the more superficial layers, e.g. the fibres of the commissure of the superior colliculus and of the radiation of Meynert.

Layer VI: Stratum griseum centrale: this is the grey matter surrounding the aqueduct of Sylvius.

The significance of the collicular stratification. Since the classic work of Cajal (1911), it has been generally recognized that only the more superficial layers of the colliculi are concerned with the reception of optic fibres.

Cajal described the fibres of the mesencephalic root of the optic tract passing first in the superficial layer of the stratum opticum. According to this author, some optic fibres terminate in this part of their course: these he termed "arborisations inférieures". Other fibres continue obliquely or sinuously towards the periphery of the colliculus, and end at varying levels in the stratum griseum superficiale almost to the plane of the stratum zonale. Fibres ending in this manner were called by Cajal "arborisations supérieures". In Golgi preparations he demonstrated that the terminal arborizations of optic fibres are rich and complex, especially those of the fibres which terminate more superficially. By means of these large arborizations, a single retinal fibre makes synaptic connexions with many cells.

Early investigators believed that the stratum zonale received fibres of retinal origin. Cajal stated that after enucleation of one eye in the mouse, Marchi sections of its brain showed no evidence that retinal fibres reached this layer of the superior colliculus. From a study of Golgi material, he concluded that the stratum zonale is made up of the axons of cells in the stratum griseum superficiale.

As a result of his exhaustive observations on the microscopical anatomy of the superior colliculus, Cajal regarded the stratum griseum superficiale, and to a lesser extent the stratum opticum, as the site of termination of the mesencephalic root of the optic tract. He was of the opinion that no fibres of retinal origin turn down into the deeper layers to end in them: and that no optic fibres end in the stratum zonale.

The work of Tsai (1925) in the opossum led to similar conclusions: optic fibres, as seen in Weigert preparations of normal brains, ended in the stratum opticum of the superior colliculus. In silver-stained material some of these fibres could be followed turning outwards to end in the stratum griseum superficiale. Using the Marchi technique after unilateral enucleation of the eye, other workers (Loepp, 1912; Brouwer *et al.* 1923; Overbosch, 1927; Bodian, 1937) have followed degenerated retinal fibres both in the stratum opticum and in the stratum griseum superficiale. In such preparations no optic fibres have been traced into the stratum zonale. The Marchi granulation is always only to be seen in the contralateral colliculus. On the other hand, Bodian (1937) has described retinal fibres ending in the anterior half of the ipsilateral colliculus of the opossum. These fibres are much less numerous than those to the contralateral colliculus, and Bodian believes that they are entirely derived from the temporal part of the retina. In addition he traced fibres showing Marchi degeneration into the stratum zonale of both colliculi, but more especially that of the ipsilateral colliculus.

Tsang (1937), with an entirely different technique, obtained comparable results in rat material. He examined microscopically the superior colliculus in Weigert sections, and by means of a screw micrometer measured the depth of the various layers. He compared normal sections with those from rats which had been deprived of one eye in infancy, and found shrinkage of the outer

three layers of the contralateral colliculus in the operated animals. In animals killed seven months after operation the depth of the stratum zonale plus the stratum griseum superficiale was 12–15 % of the total depth of the colliculus (control 19 %); similarly the depth of the stratum opticum was 20–22 % of the total (control 26 %). There was no apparent change in the depth of these layers in the ipsilateral colliculus. In the contralateral colliculus, the cells in the stratum griseum superficiale showed pronounced atrophy 7 months after operation; they were "roundish, granular, with fewer processes than normal, and with poorly stained cytoplasm". There were no recognizable changes in the cells of the stratum opticum, nor in the cells of the deeper layers. The fibres of the stratum lemnisci and stratum profundum appeared perfectly normal: the optic fibres of the stratum opticum, however, had completely vanished, though the deeper non-optic component remained intact.

The termination of optic fibres in the superior colliculus of the ferret was studied in Marchi preparations. Marchi reaction is limited to the contralateral colliculus, and is present only in the stratum opticum and to a lesser extent in the stratum griseum superficiale. No osmic acid granules are seen in the stratum zonale.

This finding is therefore in complete agreement with the observations of most other workers, but there is some reason for doubting that it is the whole truth.

A number of years ago, Prof. Le Gros Clark had cut the left optic nerve in a series of four rats in order to study early degenerative changes in the terminal fibres of the optic tract system; the animals were killed respectively 1, 2, 3 and 4 days after operation. After removal the brains were stained by a silver technique (modified Ranson method): on examination it was found that there were large granulations in the contralateral colliculus of the animals killed 2, 3 and 4 days after operation (see Pl. 1, fig. 2). These granulations represent the degenerated terminals of optic fibres: they were best shown by the rats killed 3 and 4 days after operation, and were absent in the animal killed only 1 day after operation, where the period allowed for degeneration was not sufficiently long. They are limited to the stratum opticum, the stratum griseum superficiale, and the stratum zonale: they are most prominent in the stratum griseum superficiale. The neuropil of the stratum lemnisci is more dense; however, if such granulations were present in this layer, they could not escape observation because of their large size. The contrast between the crossed and uncrossed colliculi is marked, the latter being innocent of degeneration granules. In contradistinction to the picture presented by Marchi sections this silver-stained material demonstrates conclusively that retinal fibres terminate in the stratum zonale. They are presumably the terminal branches or collaterals of myelinated fibres after the latter have lost their sheaths.

It was hoped that this phenomenon might be shown in the superior colliculus (and perhaps also in the lateral geniculate body and pretectal region) of the ferret. To this end the brains of two ferrets, after removal of the left eye, were

stained by the silver method described by Sereni & Young (1932). As it had not been possible in the rat series to demonstrate degenerated terminals in the lateral geniculate because of the great density of the neuropil, the ferret brains were sectioned at 5μ in the hope of overcoming this difficulty. The results of this experiment were disappointing, for, with the characteristic capriciousness of silver stains, the fibres of the optic tract remained unstained, although the cell-bodies and dendrites of cells in the superior colliculi and lateral geniculate bodies were well impregnated. Despite this failure it seems probable that the termination of optic fibres in the contralateral superior colliculus of the ferret is similar to that obtaining in the rat; this point must however be decided upon the result of further research.

The dual origin of the stratum opticum has been briefly referred to above. Cajal (1911) observed that, after enucleation of one eye in the cat, Marchi granulation was limited strictly to the superficial fibres of the stratum opticum. He advanced the view that the fibres of the inferior component were of cortical origin ("voie cortico-bigeminale"), and demonstrated that they ended partly in the stratum opticum itself but largely by turning downwards into the stratum lemnisci ("zone ganglionaire" of Cajal).

Barris *et al.* (1935) found that, after lesions in the striate and parastriate cortex of the cat, Marchi granules were present in the stratum opticum of the ipsilateral colliculus. In the ferret confirmation of this observation has been obtained. After removal of the occipital pole of the right side in one ferret, Marchi reaction was seen in the stratum opticum of the ipsilateral colliculus. It was not possible, however, in this preparation to determine the exact site of termination of these fibres: it appears to be similar to that of the optic component (as seen in Marchi series), i.e. in the stratum opticum itself and in the stratum griseum superficiale. The non-optic component of the stratum opticum therefore contains fibres which are derived from the visual area of the cortex: but this does not preclude there being other fibres which take origin elsewhere in the cortex.

The pretectal region

The pretectal region may be regarded as belonging to the transition zone between the diencephalon and the mesencephalon. It has been described as being composed of two nuclei, which have been designated by a variety of names by different authors. Following the nomenclature of Le Gros Clark (1932*b*), they will be called respectively the pretectal nucleus and the large-celled nucleus of the optic tract.

The pretectal nucleus. This nucleus, which may be distinguished in the brains of many small mammals as an oval mass composed of small rounded or pyramidal cells, is situated just medial to the pars posterior of the lateral nucleus of the thalamus. Caudally it enlarges and extends in a ventral direction to the medial aspect of the medial geniculate body. It corresponds to the prebigeminal (bigeminal or posterior) nucleus of Cajal (1911), the posterior

thalamic nucleus of d'Hollander (1913), Kappers (1921), and Gurdjian (1927), and the pretectal nucleus of Tsai (1925). The fibre connexions of the pretectal nucleus are many. It appears to be related by numerous short fibres to adjacent thalamic nuclei: particular attention may be drawn to its connexions with the lateral geniculate body. By way of the brachium, it is connected to the superior colliculus, and fibres from the medial lemniscus end in it. Tsai (1925) has also described fibres running to the substantia nigra (tractus pretecto-nigralis). It is traversed by fibres of passage belonging to the tecto-thalamic and cortico-tectal systems and to the mesencephalic root of the optic tract.

The large-celled nucleus of the optic tract. Along the anterior and lateral margins of the superior colliculus, dorsal and largely medial to the pretectal nucleus, is to be found the large-celled nucleus of the optic tract. Le Gros Clark (1932*b*) has homologized this nucleus with the "noyau de la voie optique" of Cajal (1911), and the nucleus parageniculatus of Lewandowsky (quoted by Loepp, 1912). It corresponds also with the rabbit "thalamus opticus" of Brouwer (1923) and the pretectal area of Gurdjian (1927). After passing over the pars posterior of the lateral thalamic nucleus, the mesencephalic root of the optic tract lies in close relation to this nucleus.

The pretectal region and the optic tract. Both the pretectal nucleus and the large-celled nucleus are in close topographical relation to fibres of the optic tract; the possibility therefore exists that optic fibres may end in them. According to the observations of Tsai, Weigert or silver preparations of the opossum's brain show optic fibres belonging to the mesencephalic root ending in the pretectal nucleus. He suggested also that fibres of the diencephalic root might reach it through the dorsal nucleus of the lateral geniculate body. Bodian (1937), in his experimental study of the optic tracts of the opossum, however, found no fibres showing Marchi degeneration in any of his material (after either unilateral or bilateral extirpation of the eye), which could be traced to the pretectal nucleus. Gurdjian (1927) believed that in the rat retinal fibres ended in the pretectal nucleus, and Lashley (1934) observed fine and rather sparse Marchi granulation in the dorsal one-third of the pretectal nucleus of the crossed side after injury to the temporal retina or after enucleation of one eye in the rat. Lashley was uncertain whether this represented terminal axons or scattered fibres passing to the colliculus.

In the rat a few degenerated optic fibres have been demonstrated by the Marchi technique passing to the large-celled nucleus (Le Gros Clark, 1931). Loepp (1912) had shown that in the rabbit optic fibres end in this region, and Brouwer (1923), experimenting on the rabbit and cat, made similar observations. Gurdjian (1927) stated that fibres of the mesencephalic root of the optic tract of the rat end in the "pretectal area" (=large-celled nucleus), and Lashley (1934) obtained fine Marchi granulation in the nucleus after unilateral enucleation of the eye in the rat, but was uncertain of its significance see above).

A review of the literature therefore establishes the fact that the large-celled nucleus receives retinal connexions, though the position of the pretectal nucleus as an optic end-station has been somewhat equivocal.

In Nissl sections of the ferret's brain, the pretectal region appears as an undifferentiated mass of grey matter; it is not possible to distinguish any discrete groups of cells which might be called the pretectal or large-celled nuclei. In silver sections the neuropil in the pretectal region is very dense. Study of such sections shows that fibres of the brachium of the superior colliculus (which is largely formed by the mesencephalic root of the optic tract) burrow ventrally, in a loop, into the medial part of the pars posterior of the lateral thalamic nucleus, and especially into that part of the pretectal region which in other species is occupied by the large-celled nucleus. In addition, a general impression is gained from silver-stained material of fibres running ventrally from the more superficial part of the pretectal region towards the region corresponding to the site of the pretectal nucleus in other animals, and also towards the nucleus of the posterior commissure. It is however impossible to trace the course of individual fibres because of the great richness of the neuropil. (It may be noted that many fibres belonging to the cortico-tectal system, after passing up along the medial aspect of the lateral geniculate, and through the pars posterior of the lateral thalamic nucleus, join with retinal fibres in the pretectal region before running up to the superior colliculus. This is clearly demonstrated by a Marchi series of the brain of one ferret from which the occipital pole had been removed.)

In two Marchi preparations of the ferrets' brains after section of one optic nerve, degenerated optic fibres are seen making a ventral loop into the pretectal region of the contralateral side before passing upwards into the stratum opticum of the superior colliculus. Marchi granulation appears mostly to be limited to the course of optic fibres of passage. No fibres showing the Marchi reaction are seen running ventrally into the deeper parts of the pretectal region, or towards the nucleus of the posterior commissure. It is impossible to state definitively whether any retinal fibres terminate in the pretectal region simply on the evidence presented by these two ferrets' brains. Because of the intimate relation of the optic tract to the region of the presumptive large-celled nucleus, and in spite of the apparent limitation of Marchi granulation to the course of optic fibres of passage, it is possible that retinal fibres—either directly or by collaterals—end in this region; on the other hand there seems to be little reason on the basis of this material for assigning to the pretectal nucleus any primary optic connexions. It should be mentioned, however, that in recent years it has been abundantly proved by two independent lines of research (S. W. Ranson and his school on the pupillo-constrictor reflex; K. S. Lashley and others on the mechanism of vision) that the pretectal region must be regarded as a primary optic end-station. Detailed account of this work lies beyond the scope of the present paper, but the appropriate sources of information will be found in the references.

DISCUSSION

Le Gros Clark *et al.* (1939) observed that gonadal activation invariably followed retinal stimulation in ferrets, even after bilateral section of the optic tracts had been attempted at the ventral border of the dorsal nucleus of the lateral geniculate body. Microscopical examination of the brains of these animals showed that some fibres of the optic tract, of one side or the other, had always escaped division, and were left in communication either with the midbrain (pretectal region and superior colliculus) or with the dorsal nucleus of the lateral geniculate body. In two animals, F 22 and F 50, these connexions were very slender; in F 22, a fine strand of fibres belonging to the left mesencephalic root remained intact, whilst in F 50, intact diencephalic fibres passed to a narrow ventrally-placed strip of the dorsal nucleus of the right lateral geniculate body (the rest of the nucleus having been destroyed by the lesion itself). Neither of these animals responded to illuminated moving objects; thus it appeared that the intact fibre connexions were non-functional so far as *ordinary* visual reactions were concerned, and it was suggested that the persistence of the gonadal response might perhaps depend upon its mediation by direct pathways proximal to, and undamaged by, the lesions.

In the present anatomical study, however, no evidence has been found to support the existence of such pathways; it seems that the ferret does not possess either dorsal hypothalamic root or accessory optic tracts, nor does the ventral nucleus of its lateral geniculate body appear to receive any optic connexions. In experiments F 22 and F 50 of Le Gros Clark *et al.*, the gonadal response of these two animals, therefore, may only have been brought about *via* the intact fibre connexions of the optic tracts. Also, since the response was obtained equally after interruption of all but mesencephalic fibres (F 22) or all but diencephalic fibres (F 50), the conception of a single special pathway may have to be abandoned.[1]

[1] It is important to record that a further series of experiments (unpublished) were carried out at Oxford during 1938–9 by Le Gros Clark *et al.*, in which attempts were again made completely to interrupt the optic tracts of the ferret, the operative technique being identical with that already described by Le Gros Clark *et al.* (1939). These experiments differed, however, from the earlier series, in that a conditioned response to light was established in the animals before operation. They were tested post-operatively for retention of the habit and retrained. At present it is only pertinent to note that amongst these ferrets was one (F 71) in which division of the optic tracts was almost complete. Microscopically, the picture presented was very similar to that of F 50; thus on the left, the lesion had successfully divided all optic tract fibres; on the right, the lesion was slightly more dorsally placed, leaving a small ventral portion of the dorsal nucleus of the lateral geniculate body (receiving diencephalic fibres) intact, though all the fibres of the mesencephalic root were cut. Gonadal activation followed retinal stimulation, though the animal appeared to be blind (as tested by methods similar to those used in F 22 and F 50). In this respect, F 71 resembled F 50. It was found, however, that the capacity of the animal to react to light by a conditioned reflex had not been abolished by the operation. In experiment F 71, therefore, it cannot be maintained that the intact fibre connexions of the optic tracts were functionless so far as other visual reactions were concerned, whilst remaining functional for the gonadal response. Hence it is open to question whether such a contention can be supported in the two earlier experiments, since it appears probable that F 50 was not completely "blind", and at least possible that F 22 was not either (though this is a more equivocal point).

One is led to further speculations on the relationship between gonadal activity and retinal stimulation. Reflexion shows that a conception may be put forward which does not demand the presence of a special pathway for the mediation of the sexual response of the ferret. It is permissible to think of the various ways in which an animal responds to retinal stimulation as different aspects of a single phenomenon, which is change of the animal's total activity induced by the stimulation. This change of total activity is a "generic" response, which may be termed "generic" visual perception.[1]

Thus differentiation of the unit phenomenon of "generic" visual perception into sharply defined categories may be necessary for descriptive purposes, but it should not be allowed to obtrude itself upon the conceptual approach to the problem. It may be stressed that this conception does not deny the existence of difference between responses to retinal stimulation, but simply affirms the unity of the underlying process, of which these differentiable responses are aspects. Gonadal recrudescence brought about by retinal stimulation may be regarded as a single aspect of "generic" visual perception (i.e. of change of total activity).

If this be so, it is not necessary to postulate that anatomical subdivision (in the sense of a special pathway) should exist; indeed, in the present paper, it has been shown that there is no direct anatomical evidence of such a pathway mediating the gonadal response of the ferret to retinal stimulation. It seems possible that gonadal recrudescence is not primarily produced by retinal stimulation, but secondarily induced by the change of total activity which follows such stimulation; in this connexion it may be noted that differentiation between stimulus and response should not be too rigidly enforced, in so far as every response itself constitutes a further stimulus.

This conception may be applied equally to stimulation of peripheral receptor groups other than retinal. Change of total activity ("generic" sensory perception) will be similarly induced by stimulation of any such group, and will become observable as a set of responses, which also need not be conceived of as belonging to entirely different categories. Grossly similar change of total activity may be obtained by stimulation of any single group, or any combination of groups, of peripheral receptors, provided that the stimulation is of sufficient intensity. It is therefore to be expected that gonadal activation (of the sexually quiescent animal) may follow stimulation of any kind. In this light, the observations of Rowan become immediately comprehensible. Reference has been made in the introduction to the early work of this author (1925) on the induction of the breeding condition in finches by retinal stimulation. He later showed (1929, 1937) that comparable development occurred in these birds if their motor activity was enhanced by a mechanical device.[2]

[1] The presence or absence of perception in an animal is judged in terms of response to stimulus: it is clear that we are not here concerned with the introspective qualities of visual perception.

[2] In these experiments the finches were placed in a cage, illuminated only by a "dim glow", which was fitted with a motor-driven travelling bar. This apparatus was turned on each evening

He also (1938*a*) published observations on precocious gonadal development in London starlings, where the operative stimulus appeared to be largely, and perhaps entirely, auditory. It may be postulated that the appearance of sexual phenomena in the ferret could be induced by substitution of some other type of stimulation for retinal stimulation. Demonstration of this would fit in with the conception outlined in this paper.[1]

It also becomes possible to understand the equivocal observations of various workers on the appearance or non-appearance of oestrus in female ferrets which had been either blinded by section of the optic nerves or kept in darkness. The potentiality for retinal stimulation in such animals is thus either completely eliminated or very markedly decreased, and this sensory loss must be reflected in their total activity (which is of course "total" in a new sense, taking into account their artificially limited potentiality for stimulation). Whether oestrus will appear in a ferret treated in either manner now depends upon the intensity of other stimulation to which the animal is exposed, which may or may not produce such a change of total activity that one aspect of the latter becomes observable as gonadal activity.

In addition to the part played by sensory stimulation (of all kinds) in the evocation of the sexual response of the ferret, other factors in its environment must be involved. For example, seasonal changes in the biochemical composition of the food, which the intact captive animal eats, must influence its total activity.[2]

Further, whether an animal is isolated from, or in contact with, its fellows must constitute another factor.

In fine, it may be concluded that any aspect of an animal's environment, if treated for experimental purposes as an isolated factor and artificially augmented to a sufficient degree, may produce changes in the animal comparable with those brought about in Nature by its environment as a whole.

SUMMARY

1. A description is given of the subcortical connexions of the optic tracts of the ferret.

2. It appears that the ferret does not possess a "dorsal hypothalamic root", or either of the accessory optic tracts.

3. In the present investigation there is no evidence that the ventral nucleus of the lateral geniculate body receives any optic connexions.

after dark for progressively increasing periods. By this means the birds were forced to move every 20 sec. The results obtained support the hypothesis—suggested above—that gonadal activation may be a response to a response; clearly in these experiments, the primary response to this method of stimulation is motor activity.

[1] Change of total activity might alternatively be shown by determination of the basal metabolic rate of the animal before and after experimental stimulation, since metabolic rate may be regarded as one facet of total activity.

[2] This is probably so, even if under laboratory conditions an attempt is made to keep the animal on a standardized diet.

4. Some evidence is presented that projection of the retina exists in the dorsal nucleus of the lateral geniculate body of the ferret, which is comparable with that described in other species.

5. It is conclusively demonstrated that, in the rat, retinal fibres end in the stratum zonale of the superior colliculus, in addition to terminating in the stratum griseum superficiale and stratum opticum; it is presumed that a similar arrangement exists in the superior colliculus of the ferret.

6. It seems probable that optic fibres end in the pretectal region, although these terminations are not demonstrable in the ferret material available.

7. The question of gonadal activation of the ferret by retinal stimulation is discussed. It is suggested that there may be no special pathway mediating this response, and that the latter may be an indirect response to the changes in total bodily activity of the animal, which are primarily induced by such stimulation.

I gratefully acknowledge the help and advice of Prof. Le Gros Clark in the preparation of this paper. I am indebted to him for suggesting the line of research, for providing most of the experimental and histological material, and for supervising the study.

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EXPLANATION OF PLATES 1 AND 2

PLATE 1

Fig. 1. F 16. Weigert-Pal. Magnification ×40. Frontal section through the thalamus of a ferret after section of both optic nerves, showing the right lateral geniculate body. This photograph illustrates well the number of undegenerated myelinated fibres (*a*) remaining in the so-called optic tracts even after bilateral section of the optic nerves. Many such fibres are present in the ventral nucleus (*v.n.*) of the lateral geniculate body; others can be seen passing over the dorso-lateral surface of the dorsal nucleus (*d.n.*). The optic tracts should not therefore be regarded as a purely retinal system. The main degenerated optic tract is seen below (*op.tr.*).

Fig. 2. Rat 15. Modified Ranson. Magnification ×740. Frontal section through the contiguous borders of the two superior colliculi of a rat killed 72 hr. after section of one optic nerve. To the left of the middle line large black granulations are seen in the colliculus of this side. They are absent in the other colliculus. These granulations represent the degenerated terminals of crossed retinal fibres, and are present even in the stratum zonale.

PLATE 2

Figs. 3, 4. F 59. Marchi. Magnification ×29. Frontal sections through the middle of the right (contralateral) and left (ipsilateral) lateral geniculate bodies; Marchi reaction appears in both the ventral and dorsal nuclei, but in the former it is confined to fibres of passage. For interpretation, see text.

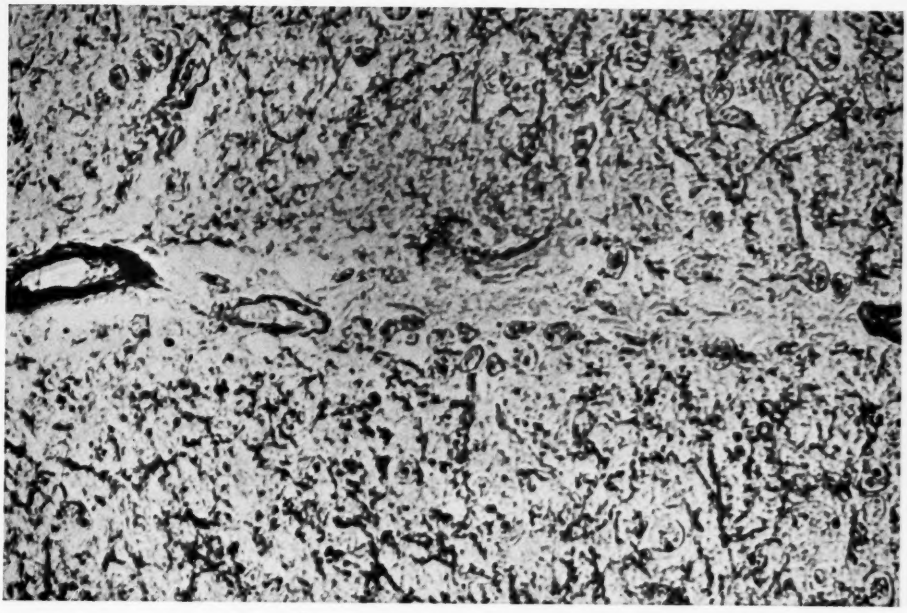

Fig. 2.

Fig. 1.

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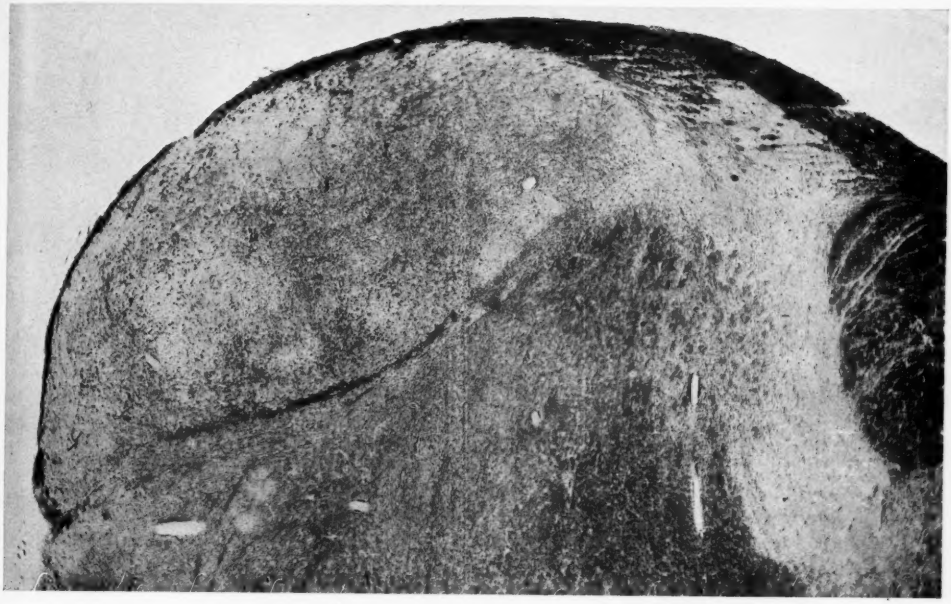

Fig. 4.

Fig. 3.

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ANATOMICAL NOTES

THE AMOUNT OF MUCOSAL TISSUE IN THE SMALL INTESTINE

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Some time ago our colleague, Prof. B. P. Babkin, of the Department of Physiology, McGill University, asked if we could furnish him with exact information as to the amount of mucosa or secretory tissue in the small intestine. A search of the recognized text-books and literature failed to supply the required information, although, as pointed out by Dr Babkin, it was available in part in the case of the dog. Abel & Kubota (1919) stated that in a dog weighing 20·14 kg. they removed, opened, washed and dried between sheets of filter paper the entire stomach and small intestine, and then scraped off the mucosa with sharp pieces of glass: they found that the scrapings weighed 242 g., while the residual muscular part of the alimentary tract weighed 335 g. Apart from this paper we could find no reference to the weight of the mucous membrane of the small intestine.

Accordingly, through the kind co-operation of the Department of Pathology of McGill University, we obtained the entire small intestine of six adult men who had died from causes that had no connexion with the alimentary canal. The mesenteries and all fat were carefully dissected off these specimens and they were then opened and thoroughly washed out. To preserve them, they were placed in a solution of 5 % formalin with a little glycerine and some alcohol. The duodenum, jejunum and ileum were separated, each was pinned down on a cork mat and the entire mucous membrane was dissected away from the muscular layers—a tedious but quite feasible operation which left both the mucous membrane and muscular layers as continuous sheets. The sheet of mucous membrane still retained its circular folds, although, unlike the muscular layer, it stretched considerably as it was detached.

Both sheets were then pressed firmly by hand between thick sheets of blotting paper and weighed. The results are set out in Table 1. It is difficult to decide exactly how much drying by pressure should be carried out; the mucosa especially can contain a considerable quantity of fluid, and how much of this should be regarded as a normal constituent of the tissue was uncertain. Accordingly, we weighed the last two specimens again after subjecting them to heavier and more prolonged pressure. They were laid out in a single layer between dry towels and placed in a letter-press, where for 10 min. they were tightly squeezed. They were then placed between thick sheets of blotting paper and again squeezed in the press for a further 10 min. The results of these second weighings are shown at the foot of the table, but we think that these weights should be disregarded as they were obtained after subjection of the tissues to a much severer drying process than is usually adopted in estimating the weight of viscera. In one specimen the complete pancreas was also present and as a standard of comparison we give its weight after gentle drying with a cloth.

In only one specimen (no. 5) did we find any recognizable pathological lesion, but we consider that no. 6, in which the weight of the mucous membrane in the jejunum and ileum was far in excess of that found in the five other examples, should be regarded as abnormal and possibly pathological. In this specimen the mucous membrane was extraordinarily thick and yellowish in appearance, but the mesenteries and outside of the intestine contained little fat.

Our results show that the weight of the entire small intestine and that of its mucosa vary over a wide range. The weight of the mucous membrane, excluding

specimen 6, varies from 72·46 to 243·9 g., the average being 166·35 g. In the duodenum and jejunum the mucous membrane makes up almost two-thirds of the total weight of the intestine, whereas at the lower end of the ileum it barely makes up one-third. The weight of the mucosal tissue in the small intestine is therefore considerable and appears to be appreciably in excess of the total weight of the pancreas. Of course, the mucous membrane, as dissected off by us, contains much more than pure secretory tissue—blood vessels, lacteals and the muscularis mucosa are all included—but the pancreas and the salivary glands, as usually weighed, likewise contain considerable amounts of vascular and supporting fibrous tissue.

Table 1. ***Weight (in grams) of the mucosa of the small intestine in six adult male human subjects***

	Total weight of small intestine	Total weight of		Total weight of mucosa in			Relative weight of mucosa to muscle (weight of muscle in each case being taken as 1·0)			
		Muscle	Mucosa	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum	Total small intestine
				Hand dried						
(1)	270·50	115·50	157·00	19·00	107·00	31·00	1·77	1·72	0·74	1·34
(2)	413·20	169·30	243·90	29·60*	125·00	89·30	2·00	1·18	1·12	1·33
(3)	250·43	93·85	129·25	21·32	76·51	31·42	2·00	1·58	0·90	1·36
(4)	135·41	62·95	72·46	12·53	40·80	19·13	2·17	1·60	0·60	1·15
(5)†	459·94	230·80	229·14	35·00	166·74	27·40	1·33	1·08	0·55	1·00
(6)‡	474·07	129·94	344·13	25·74	217·73	100·66	1·90	3·88	1·67	2·64
				Dried by press						
(5)†	373·84	194·10	179·74	35·00	121·74	23·00	1·33	1·00	0·50	0·81
(6)‡	340·25	82·59	221·66	16·40	133·66	71·60	1·80	3·66	1·90	2·56

* This figure is doubtful, as the material became accidentally over-dried and had to be remoistened.

† Two small ulcers were found at the upper end of the duodenum in this specimen and the blood vessels in the vicinity were thrombosed.

‡ In this specimen the total weight of the pancreas, after gentle hand-drying, was 62·13 (the pancreas was not available in the other cases).

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THE APPLICATION OF TRICHROME STAINING METHODS TO EMBRYOLOGICAL TECHNIQUE

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This paper summarizes the results of seven years' experience with trichrome staining methods in the preparation of several mammalian embryonic forms (including the human) for teaching and research. I have adapted the original methods of Masson (1929) and Heidenhain (1915) for these purposes and the modifications are described below.

Many embryologists seem to be deterred from the use of trichrome stains because they consider them complicated and time-consuming; also, these disadvantages seem to be accentuated by the fact that in their work large series of sections must be stained. My experience, however, is that these objections are more apparent than real; there are only a very few stages in these staining methods where individual treatment of the slides is essential, and the beauty and clarity of the finished sections more than compensate for the slight additional time required for their preparation.

Preliminary treatment of embryos

Several points in the preliminary treatment of the material are of special importance. A number of fixatives have been tested and, of these, I have found Bouin's fluid to be excellent as a preliminary to the Masson staining methods. Formol-bichromate and Zenker-formol are also fairly good. With the Heidenhain method, on the other hand, the best *staining* results have been found with fixation in a fluid containing mercuric chloride; but a greater or lesser degree of shrinkage takes place. Fixation in 10% formalin has not been found to be satisfactory for this type of work. I have had no experience with the Presbyterian Hospital technique (Lillie, 1938), in which formalin-fixed material is mordanted upon the slide with Bouin's fluid. In view of the results reported, this method might be worthy of trial with formalin-fixed embryos.

Most of the embryos used in this study required to be decalcified, and, after a number of trials, I now use as a routine measure, when necessary, 2% nitric acid in 70% alcohol. The action is quite slow (a 35 mm. rabbit embryo requires 7–8 days for thorough treatment, the fluid being changed every two days). The optimum time may be exceeded without untoward results if later the embryo be washed very thoroughly in 70% alcohol to which a small quantity (0·25%) of potassium alum has been added. This counteracts the slight swelling effects of the nitric acid.

In the further treatment of these embryos my aim has been to combat shrinkage as far as possible, and at the same time to preserve the mutual relations of the embryonic structures. Double embedding in celloidin and paraffin has proved undoubtedly the best method for the preparation of serial sections of embryos of which I have had experience. Sections are cut upon a sliding microtome (using the water-on-the-knife method of Huber, as elaborated by Heuser (McClung, 1929)). The procedure admittedly takes more time than simple paraffin embedding, but the shrinkage is much less and the tissue-relationships are infinitely better preserved.

The dioxan-paraffin method as used by Mossman (1937) has been found of service. Dioxan has been substituted for the higher concentrations of alcohol in dehydration and has been used also as a vehicle in the transition of embryos from alcohol to paraffin. Shrinkage is rather more marked than when double embedding is used.

With ordinary paraffin embedding methods shrinkage has been found difficult to combat and the distortion due to unequal expansion of the paraffin sections upon the slide during spreading has later caused complications in reconstruction. I have also tried the methyl benzoate-celloidin-paraffin method (Romeis, 1932) but I have not found the results satisfactory.

Modified trichrome staining methods of Masson

Masson (1929) described a number of trichrome staining methods. Of these, I have found the iron-haematoxylin : ponceau-acid fuschin : light green technique most suitable for embryos.

In order to economize in time and labour during the staining of slides, glass staining troughs and racks are very helpful. I mount my embryological sections usually on 3×2 in. slides; so troughs and racks of the following dimensions are most useful: troughs $10 \times 8 \times 7$ cm., racks $8{\cdot}5 \times 6{\cdot}5 \times 3$ cm. Each rack is grooved to accommodate 10 slides. (20 slides arranged back to back *can* be accommodated in one staining rack, but this is not recommended.)

Steps of the method

(1) Xylol.

(2) Absolute alcohol. If the embryo has been doubly embedded in celloidin and paraffin, pass the slides through two baths of equal parts of absolute alcohol and ether. The object of this is to remove as completely as possible the original celloidin mass used for embedding. I have found that if the original celloidin used for embedding be not fully removed a very undesirable precipitation of stain upon the slide occurs later.

(3) 0·5 % celloidin dissolved in equal parts of absolute alcohol and ether. This fresh celloidin coating is necessary if hot staining solutions are subsequently used.

(4) Remove slides, drain off all superfluous fluid, allow partly to dry in the air and then immerse in 80 % alcohol.

(5) 60 % alcohol.

(6) 30 % alcohol.

(7) Distilled water.

(8) Mordant sections in 5 % iron alum heated to 45° C. for 5 min.

(9) Wash with tap water for 2–3 min.

(10) Stain in Regaud's haematoxylin heated to 45° C. for 5 min.

(11) Place the staining rack and slides in distilled water.

(12) *Decolorization* is carried out in picric acid-alcohol (as recommended by Masson (1929)). Masson states that this fluid has a rapid action. My experience with embryos has been quite different. Decolorization is usually slow and delicate, which is a virtue. In many instances I have found that 20 or 30 min. may elapse before sections are properly affected. My custom is to take one slide from each stained batch and study the progress of decolorization under the microscope. This then gives a definite idea of the time necessary for treatment of that batch.

The remainder are treated together and the process is stopped just before the terminal stage by immersion in tap water. Final differentiation of each in picric acid-alcohol is then checked by direct observation under the microscope, which takes merely a few minutes.

Very rarely one finds an embryo where the excess of haematoxylin is rapidly removed by picric acid-alcohol. When the test slide of a series shows this, the remainder should be treated with a weaker decolorization solution (such as 1 part of the picric acid-alcohol to 2, 3, or more parts of 95 % alcohol). *This is the only step in this method where individual treatment of the slides is necessary.*

(13) Wash the slides thoroughly in running tap water (30 min. at least). This removes all traces of picric acid from the tissues, which is very important, and this

removal may be accelerated by passing the slides into 70 % alcohol for 10 min. or so and then back to water.

After differentiation, all nuclei should be clear blue against an almost colourless background. Incomplete differentiation affects the further steps in the method.

(14) Distilled water for 5 min.

(15) Diluted ponceau-acid fuchsin stain—1 hr. Details of the preparation of this stain are given by Masson (1929). I have found the proportion of acid fuchsin in his original mixture too strong for embryos and my best cytoplasmic staining results have been obtained by immersion for 1 hr. in 4 parts of the original ponceau de xylidene stain added to 1 part of the acid fuchsin stain, the resultant mixture being then diluted ten times with 1 % acetic acid.

(16) Rinse in distilled water for 1 min.

(17) Immerse in 1 % aq. phosphomolybdic acid for 5 min.

(18) Wash in distilled water for 1–2 min.

(19) Stain now in a 2 % solution of light green[1] in 1 % acetic acid for 3 min.

(20) Pass directly into a bath of 1 % acetic acid for 2 min.

(21) 95 % alcohol for 2 min.

(22) Absolute alcohol for 2 min.

(23) Absolute alcohol-ether (equal parts) until the celloidin pellicle is dissolved.

(24) Xylol.

(25) Mount in salicylic acid balsam.

Some further notes may be helpful to other workers. Slides stained by this method seven years ago seem as brilliant to-day as they were when first made. No particular care has been taken to avoid exposure of these slides to excess of light.

Fast Green F.C.F.[2] has been tried as a substitute for light green in this method. It is much more powerful than light green; 0·5–0·75 % solution in 1 % acetic acid is the correct strength in which to employ it. Collagenous tissue after this stain is blue-green in colour and is especially noticeable after formalin fixation.

Modified azan stain of Heidenhain

I have mentioned above that the optimum results with this stain are obtained after fixation in a fluid containing mercuric chloride. Susa's fluid and mercuric chloride-acetic acid are the best of these. Again in this method I use staining troughs and racks as described in the other.

Steps of the method

The sections are brought to water in the same manner as in the previous method, but since fixation has usually been in a fluid containing mercuric chloride they are treated with a weak solution of iodine in 70 % alcohol for 5 min. to remove any sublimate crystals in the tissues. The excess of iodine is then removed by immersion for a short time in a bath of 50 % alcohol, containing 0·25 % sodium thiosulphate. Then the procedure is as follows:

(1) Stain the sections in azocarmine for 45 min. at 55° C. Allow to cool for 10–15 min. Details of the preparation of the azocarmine stain are given in the text-book of Romeis (1932, p. 413). I have used azocarmine G in 0·1 % solution.

(2) Rinse in distilled water.

(3) Differentiate in anilin-alcohol (anilin oil 1 c.c. in 1000 c.c. 90 % alcohol). This differentiation is important and must be controlled under the miscroscope. The object is to extract the stain from the tissues until the nuclei are sharp and of a

[1] The light green stain which I have hitherto used is described as "Lichtgrün F.S. Yellowish", and was obtained from Dr G. Grubler and Co., Leipzig. The light green stain manufactured by many American firms, and certified by the Stain Commission, would serve equally well.

[2] Fast Green F.C.F. (dye content 94 %), certification number N.G.f. 2.

deep red colour. It is often helpful to dilute the anilin-alcohol solution with distilled water.

When the sections have to be examined under the microscope, interrupt the process of differentiation by dipping the slide several times in acetic-alcohol (prepared as in stage 4). If it has not reached the desired stage, continue by transferring the slide again to anilin-alcohol, re-examining until the extraction of stain is satisfactory.

(4) Wash for a short time in acetic-alcohol (glac. acet. acid 1 c.c. in 100 c.c. 95 % alcohol) to stop the differentiation.

(5) Immerse in 5 % aqueous solution of phosphotungstic acid for 1 hr.

(6) Wash quickly in distilled water.

(7) Stain in the following mixture for 30 min.: orange G. 2·0 g., anilin blue (water soluble) 0·5 g., glac. acet. 8 c.c., distilled water 100 c.c. Before use, dilute this with three times its volume of distilled water.

Fast Green F.C.F. has been substituted in this staining mixture for anilin blue. The results have been very satisfactory but 0·35 g. Fast Green must be used instead of 0·5 g. anilin blue.

(8) Wash for a very short time in distilled water.

(9) Differentiate in 95 % alcohol. The slides are examined individually under the microscope at this stage. One must bear in mind that further slight extraction of the anilin blue-orange G stain will occur in the next stage.

(10) Absolute alcohol.

(11) Xylol.

(12) Mount in balsam.

This azan staining method gives very beautiful results; but it must be stressed that fixation is important, a fixative containing mercuric chloride being recommended. Also, the differentiation of the azocarmine stain with anilin-alcohol has to be carefully controlled.

Discussion

I have described two trichrome staining methods which have proved in my hands very satisfactory for the study of mammalian embryos. It must be remembered that these embryos were those in which epithelial and connective tissue differentiation had commenced. Young embryos, on the other hand, are best studied after the use of an iron-haematoxylin nuclear stain followed by a simple cytoplasmic counterstain (such as orange G in 1 % solution).

Trichrome stains definitely require more care in their application and consume more time than simple embryological stains. I am strongly of the opinion, however, that more use should be made of these methods by embryologists who work upon the later stages of development.

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REVIEWS

Organizers and Genes. By C. H. WADDINGTON. (Cambridge University Press.) 1940. Price 12*s.* 6*d.*

During the past thirty years the chromosomal theory of heredity has been firmly established and there is now a considerable body of knowledge concerning the ultimate chromosomal constituents, the genes, and their influence on hereditary characters. During the same period experimental embryologists, by the development and elaboration of new technical methods and the strict application of non-vitalistic causal principles, have revivified the study of development, more particularly of its earlier stages, and have established the concept of organizer activity in embryological processes. How genes produce the hereditary effects or how organizers organize developing tissue are still almost untouched problems. That the basis of the mechanism in each case is the production or liberation of a chemical substance which has an activating or inhibitory effect on protoplasmic metabolism, or which changes the physico-chemical properties of protoplasm, seems almost certain, but the nature of such substances and the mechanism whereby they produce their effects are almost completely unknown. Analogy with the better-known hormones of adult organisms, or with vitamins, is helpful in providing a schematic idealization of the gene and organizer processes but, in the present state of our knowledge, it can be no more than analogy. Even when the substances have been isolated we shall still have the problem of the reactivity of the tissues, the cells, and the physico-chemical systems influenced by them, a problem heretofore largely ignored by endocrine physiologists.

In the book under review Dr Waddington has summarized his personal views and his own investigations on these problems, and he shows that there are considerable resemblances between gene and organizer activity. Dr Waddington is one of the group of Cambridge biologists which has done so much to rehabilitate British embryology, and to remove it from the rut of detailed morphological description and place it on the more promising pathway of causal analysis. His views, then, demand sympathetic attention. A reading of this book, however, leaves one with mixed feelings. This is, in part, due to the complicated nature of the problems involved. As has already been stated, our present state of knowledge is not such that it allows of a satisfying statement of either organizer or gene mechanism of action; still less, therefore, of a synoptic consideration of both together. Then the book is too brief for the importance of the subject-matter and it has not been recognized sufficiently that most embryologists are not specialist geneticists nor, I presume, will geneticists be able, readily, to follow the embryological side of the argument. Terms, some new, abound; Dr Waddington has accused Balinsky of tautology in reference to the latter's interpretation of induction as a raising of the metabolic activity of the tissue on which the organizer acts. Here, doubtless, logicians would agree, but one has the feeling that a Wittgenstein would soon find a number of tautologous statements in Dr Waddington's book. Indeed some seem obvious to one untrained in critical logic, e.g. "development will appear as a result of a complex of reactions between substances which form an unstable mixture, which may at certain times have two or more alternative modes of change open to it", or again, "the interaction of two autocatalytic substances may explain the frequent periodic phenomena of development, such as the formation of somites and other segmental organs".

The book finishes with an appeal, which some may find gratuitous, but which the reviewer thinks eminently reasonable, for the recognition of the importance of a philosophical attitude in science. The urgent need in biology, though, is not for an all-encompassing scheme, be it dialectical materialism, which Dr Waddington suggests is more reasonable than most, or otherwise, but for a critical approach to the problem of terminology. The seventy-and-seven forms of ambiguity should be cleared up. In a sense Dr Waddington's book is helpful in this regard; he obviously feels uncertain about certain current terms and is groping towards new ones, or, at any rate, better defined ones. The introduction of the field concept into embryology has made this imperative lest we find ourselves with a terminology which explains phenomena by the old and stultifying trick of giving them a name. Finally one must protest against the topological interjection. Dr Waddington himself seems to have realized its inadequacy by a half-hearted apology for its introduction. Nothing could be more praiseworthy than Dr Needham's recent insistence on the necessity of applying the mathematical apparatus of *analysis situs* to the study of morphogenetic change, but the casual introduction of what can only be called a "gesture" formula, which can have no significance for most biologists, is unworthy of the importance of this possible approach.

Dr Waddington's book cannot be regarded as a prolegomena to any future embryology; indeed it is often in the nature of a collection of *obiter dicta*; but it is interesting, and often illuminating, and is a good pendant to Dr Needham's entrancing Terry Lectures published under the title "Order and Nature". J. D. B.

The Anatomy of the Eye and Orbit: Including the central connexions, development, and comparative anatomy of the visual apparatus. By EUGENE WOLFF, M.B., B.S. (Lond.), F.R.C.S. Second edition. Pp. x + 374. 242 illustrations. Price 31*s.* 6*d.* Published by H. K. Lewis and Co., Ltd., 136 Gower St, London, W.C. 1.

One of the outstanding features of the first edition of Mr Wolff's text-book was the excellence of the illustrations, whether drawings or photomicrographs. The second edition has been embellished with sixty additional illustrations of equal distinction, and both the author and the publishers are to be congratulated on a very fine production. As a student's text-book, the volume is particularly attractive, for it is written in an easy and direct style which allows of ready assimilation. If we were pressed for any specific criticisms, we would confess to a little disappointment that the section on the central connexions of the retina has not been brought more up to date. For example, it is now accepted that there is no evidence that the transverse peduncular tract in man (if it exists in the human brain) contains optic fibres; there is no real evidence that geniculo-thalamic fibres exist in any mammal; no reference is made to the recent work concerning the site of termination of crossed and uncrossed fibres in the lateral geniculate body, the precise nature of the retinal projection in the visual cortex, or the importance of the pretectal nucleus as a pupillary centre; it is not the case that in marsupials there are no uncrossed retinal fibres; the so-called ventral nucleus of the lateral geniculate body is probably not a visual centre at all. In the section on comparative anatomy, there are occasional statements which require qualification, e.g. the statement that the change from bigeminal to quadrigeminal bodies is well seen in amphibians, and that the cortex is smooth in edentates, rodents, and some monkeys.

However, it is not to be doubted that this book will certainly require a third edition, for its popularity is well assured. This will provide the opportunity for any further revision which may be desired.

A General Zoology of the Invertebrates. By G. S. Carter. Pp. ix + 509. 172 illustrations. Price 25*s*. Published by Sidgwick and Jackson, Ltd., 44 Museum St, London, W.C. 1.

For the study of fundamental problems of living structure, anatomists have frequently to turn their attention to invertebrate organisms, since the latter provide many opportunities for the investigation of animal protoplasm and cellular organization which are not to be found among the vertebrates. Anatomists, therefore, will be well advised to direct their attention to this book, and particularly to the first three sections, dealing respectively with protoplasm and the free-living cell, the multicellular body, and comparative physiology. The book deals with its subject in a general manner, and the author gives a very clear account of the organization and physiology of invertebrates. As an introduction to a dynamic study of zoology it is unusually effective.

A Method of Anatomy. By J. C. Boileau Grant, M.B., Ch.B., F.R.C.S. Second edition. Pp. xxii + 794. 651 illustrations. Price 33*s*. Published by Baillière, Tindall and Cox, 7 and 8 Henrietta St, London, W.C. 2.

A thoroughly refreshing book on topographical anatomy, written in a clear, crisp style, and replete with clear, crisp diagrams. The body is dealt with regionally, and Prof. Grant makes full use of the deductive method of reasoning in the presentation of the many details of topographical relationships with which the medical student must necessarily become acquainted. *A Method of Anatomy* raises only one regret in the mind of the reviewer—it is that such a book was not available when he himself was first studying human anatomy in the dissecting room.

Cunningham's Manual of Practical Anatomy. Revised and edited by J. C. Brash and E. B. Jamieson. Tenth edition. (London: Oxford University Press. Oxford Medical Publications.) 1940. In three volumes. Vol. I: General Introduction; Upper Limb, Lower Limb, pp. xix + 411, 185 figs. Vol. II: Thorax and Abdomen, pp. x + 500, 235 figs. Vol. III: Head and Neck, Brain, pp. x + 535, 234 figs. Each volume, Price 15*s*.

We heartily welcome the jubilee of this great work. Those who have grown up with a book are naturally conservative and tend to resent changes, but there are some innovations, which first appeared in the last and are still adhered to in this edition, to which we would earnestly draw attention. First, the rearrangement of the order of dissecting the head and neck seems to us to have no advantages, but rather increases the students' troubles in tackling this most difficult part. In a few words, it means that its dissection is done for the most part from above downwards instead of in the reverse direction.

Secondly, the method of dissecting the pelvis from the side, whereby the innominate bone is first removed, is in our view too elaborate and difficult a procedure for the beginner. The older plan of sectioning the pelvis is at once simpler and equally informative and, where in these days several students may have to share the part, permits each half of the group to have a moiety of the pelvis to themselves. Again, instead of keeping the description of the male and of the female generative organs in separate sections, the accounts of both are mixed up together. This seems to be pointless, and it has the great disadvantage that the student, who can only be expected to be dissecting either a male or a female pelvis at any one time, has to pick the pages, which concern him immediately, here and there.

The author of this book was a master teacher, and the methods he originally gave have stood the test of years of trial in dissecting rooms the world over. We would, therefore, beg the present editors to reconsider these matters in a future edition. Otherwise there is nothing but praise for the high standard which is still maintained.

A Manual of Embryology. The Development of the Human Body. By J. Ernest Frazer, D.Sc., F.R.C.S. Second edition. (London: Baillière, Tindall and Cox.) 1940. Pp. x+523. 282 figs. Price 30*s*.

The author is to be much congratulated on the fact that a second edition of this work is called for. The book is a veritable monument to his life's work, and, even if certain of its interpretations are contrary to those of other observers, it should continue to attract readers, both students and teachers, in increasing numbers by its sincerity, accurate description and clear illustration. There are few important changes in the new edition. A fresh section has been added on the foetal circulation, and such alterations as have been made in the arrangement of figures and text all go to enhance its value.

CELL COLUMNS IN THE SPINAL CORD OF A HUMAN FOETUS OF FOURTEEN WEEKS

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INTRODUCTION

The cell columns of both normal and abnormal human spinal cords have been described by many authors, but this literature does not include many examples of early foetal stages. Researches which have been carried out by the present author to determine the development of the cell columns in the cervical region of the spinal cord of the rabbit have shown that at certain stages in their development the cell columns are much more distinct than in the adult animal. This is particularly true of the early foetal stages in the rabbit. In the case of this mammal no evidence has been found to confirm the statement of Angulo y Gonzalez, with reference to the white rat, that the cell columns are more numerous and complex in the newborn than in the adult, but he has probably come to this conclusion because of the difficulty of determining the cell columns in the adult as compared with the earlier stages when they are much more compact and discrete. It seemed of interest, therefore, to determine the cell columns in the cervical and lumbar enlargements of the spinal cord of a well-fixed human foetus of fourteen weeks, for this might give a clearer picture of them than the later stages.

METHODS

The spinal cord was removed, together with the roots and ganglia, after fixation in 10% formalin, but, since this fixative does not allow satisfactory staining with toluidin blue in the case of embryos and foetuses, the cord was again fixed in acid alcohol (which has been found to improve the staining properties of the nerve cells in the spinal cord of embryos fixed in formalin). The cord was then dehydrated, embedded in paraffin wax and serially sectioned at 15 μ. The sections, mounted on slides, were first stained with toluidin blue. This series was carefully studied and serial drawings and photomicrographs made to show the main cell columns in the spinal cord. The sections were then destained, and restained by the Bodian protargol method to show the fibres with a view to determining if possible the connexions of the cells in some of the columns, and to make certain that none of the divisions described in the toluidin blue series was due simply to the passage of a bundle of fibres through a cell column.

NOMENCLATURE

The columns in each of the cervical and lumbar series are numbered in order of their appearance when the series is traced caudally from the cephalic part of the enlargement. No names have been used to designate the cell columns of

the enlargements, for, although this method has the advantage of describing the position of a cell group, it becomes too complicated when the number of groups increases beyond five or six. From this it will be seen that the numbers given do not necessarily correspond with the numbers used by other authors; identical numbers might cause homologies to be drawn between cell columns of the same anatomical position though not necessarily of similar function in different animals.

THE CERVICAL ENLARGEMENT

(Text-fig. 1; Pl. 1, fig. 1)

In the lower part of the third cervical segment[1] there exist four cell columns which have been numbered from medial to lateral 1, 2, 3 and 4. Column 1 lies at the anteromedial aspect of the anterior horn with column 2 situated just posterior to it and in some sections fused with it. Column 3 lies at the anterolateral edge of the anterior horn just lateral to column 2, while just posterolateral to this is column 4. Traced caudally, columns 1 and 2 become completely fused in the fifth cervical segment, and the fused mass, consisting mainly of cells of column 1, remains in the same position throughout the cervical enlargement.

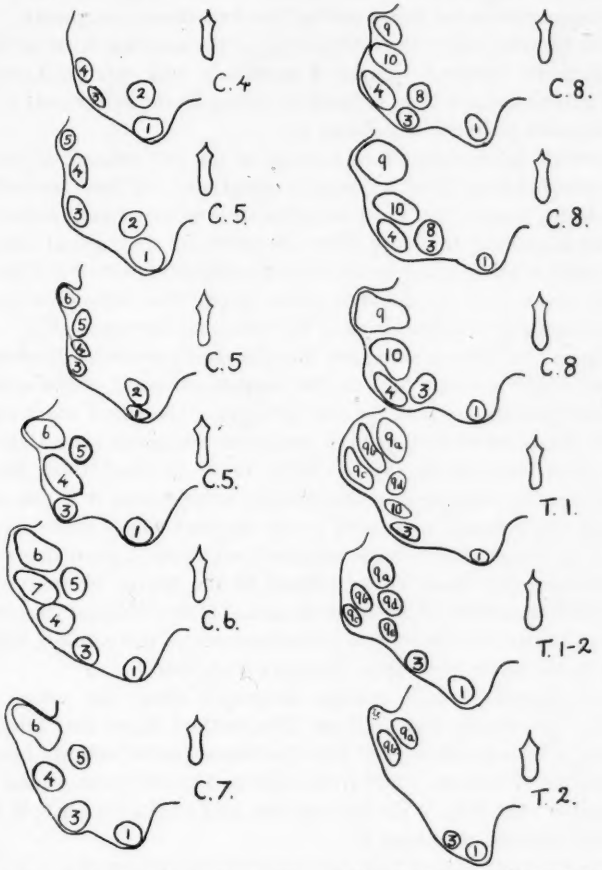
Column 3, traced caudally, retains the position described above till, passing medially in the eighth cervical segment, it fuses with the anterior surface of column 8 forming an anterior column which, decreasing in size, inclines medially in the first thoracic segment towards column 1, on whose lateral aspect it enters the upper part of the thoracic spinal cord.

Column 4, which lies posterior to column 3 in the fourth cervical segment, expands in a posterior direction throughout the fifth, finally dividing into a dorsal and a ventral part in the sixth. The dorsal part, column 7, is only present throughout the latter segment and becomes fused in its caudal part with column 4 which, extending down into the eighth cervical segment, again divides into anterior (column 4) and posterior (column 10) parts. The former fades out in the upper part of the first thoracic segment, having maintained a posterolateral position throughout. Column 5, which appears posterior to column 4 in the upper part of the fifth cervical segment, rapidly divides into posterolateral (column 6) and anteromedial (column 5) parts. The latter passing anteromedially to take up a central position in the sixth cervical segment is continued caudally in this situation and disappears in the upper part of the seventh. Column 6 rapidly increases in size throughout the fifth and sixth cervical segments and is situated here posterior to columns 4 and 7. It disappears in the upper part of the eighth cervical segment where column 9 overlaps the posterior part of its caudal extremity. Column 8 appears medial to column 4 close to the caudal end of column 5. From this central position in

[1] Unfortunately the first three cervical segments are missing from the spinal cord, these having been removed with the head for a previous study.

the seventh cervical segment it inclines anteriorly to unite with the posterior surface of column 3 in the eighth cervical segment.

Column 9, which is the most posteriorly situated cell column of the anterior horn from the eighth cervical to the upper part of the second thoracic segment,



Text-fig. 1. A diagram to show the relative positions of the cell columns of the cervical enlargement of the spinal cord of a 14-week human foetus. Where more than one drawing of a particular segment is made the first drawing shown is the most cephalic in this figure and in Text-fig. 2.

is single in its cephalic part but, after considerable expansion, becomes divisible into subsidiary columns from the first thoracic segment caudally. These subsidiary columns are irregularly fused and are therefore considered to be divisions of column 9 rather than separate cell columns. Four parts of this cell column are visible in the first thoracic segment: three (9*a*, 9*b*, 9*c*) lie at the

lateral edge of the column, one behind the other, so that 9*a* is the most posterior, while the fourth (9*d*), situated medial to them, lies posterior to column 10. Further caudally a fifth part (9*e*), lying anterior to 9*d* and medial to 9*c*, is occasionally present. Of these divisions only columns 9*a* and 9*b* extend into the upper part of the second thoracic segment, 9*c*, 9*d* and 9*e* having disappeared in the lower part of the first thoracic segment.

Column 10, situated at the lateral edge of the anterior horn in the eighth cervical segment between column 4 anteriorly and column 9 posteriorly, extends further caudally than column 4, ending in the upper part of the first thoracic segment posterior to column 3.

The cervical enlargement thus consists of ten cell columns of which only two are continued down into the thoracic spinal cord. Of these ten cell columns column 9 is the largest and most complex; it is situated most posteriorly and passes further caudally than any other cell column of the cervical enlargement, its caudal part coming into relation with the cephalic part of the intermediolateral cell column of the thoracic spinal cord. This column 9 reaches its maximum degree of differentiation in the first thoracic segment.

There is much evidence to suggest that the most posterior cell column of the lateral part of the anterior horn in the caudal extremity of the cervical and lumbar enlargements contains the cells of origin of the axons which supply the muscles of the hand or foot in man and other mammals (van Gehuchten & de Buck, 1898; van Gehuchten & Nelis, 1899; de Neef, 1901; Bok, 1928; Yu-Ch'üan Tsang, 1939), and in the cervical enlargement it is not surprising to find that this column is situated in the segment whose nerve supplies the muscles of the hand, and that the column itself shows a great degree of complexity which seems likely to be related to the degree of anatomical and functional differentiation of the hand in man. (It is of interest that the lateral extension of the anterior horn, due to the presence of this column, is greater in man than in the anthropoid apes; Kappers *et al.* 1936.)

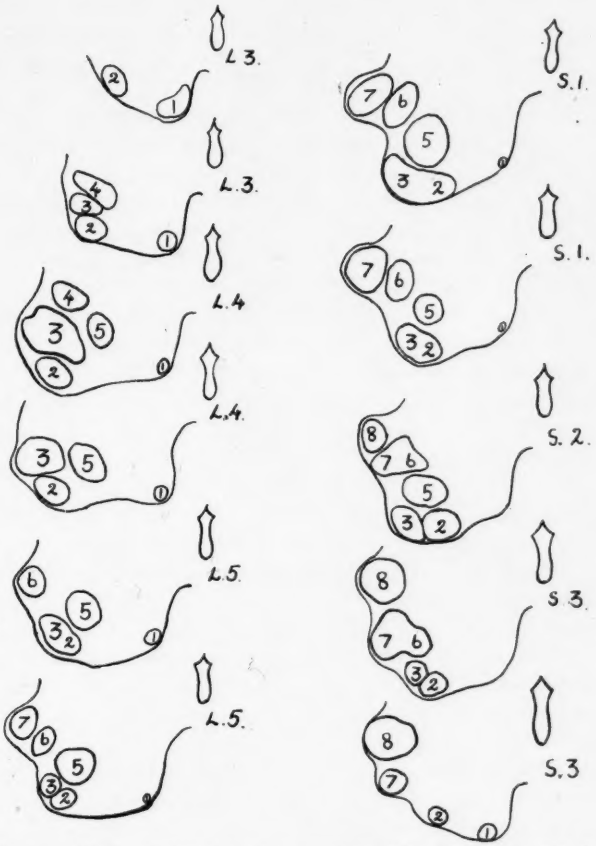
Sections stained by the Bodian technique show the same columnar structure as the toluidin blue sections. This method shows that fibres passing to the spinal accessory nerve arise from the dorsal part of column 4 cephalic to the appearance of column 5 and from column 5 in the more caudal part. It seems possible that this is all one column and that column 5 is merely a disconnected portion of column 4.

THE LUMBAR ENLARGEMENT

(Text-fig. 2; Pl. 1, fig. 2)

In the lower thoracic region a mass of cells situated at the anterior part of the anterior horn can be divided into anterior, central and anterolateral groups all of which are intimately connected with each other. In the upper lumbar region the central and anterolateral groups disappear but the anterior group is carried caudally into the lumbar enlargement and is called column 1.

Column 1, which is a small group of cells, retains the position described above throughout the lumbar region but decreases in size and disappears in the first sacral segment; it reappears in the third and again disappears in the fifth. Column 2, arising in an anterolateral position in the second lumbar segment,



Text-fig. 2. A diagram to show the relative positions of the cell columns of the lumbar enlargement of the spinal cord of a 14-week human foetus.

increases rapidly in size becoming divisible into anterior (column 2) and posterior (column 3) parts, which continue caudally close together in a similar position and coalesce in the fifth lumbar segment. From this position the single column extends into the third sacral segment where it disappears. Throughout its length column 2 is characterized by the presence of a bundle of longitudinal fibres running in its substance.

Column 4, appearing as a cap of cells over the posterior aspect of column 3 in the third lumbar segment, expands medially and splits into a medial (column 5) and a lateral (column 4) part. The latter fades out in the middle of the fourth lumbar segment dorsal to column 3.

Column 5 sweeps further anteromedially to become central in position in the fourth lumbar segment, where it is divisible into an anterior and a posterior part by virtue of the presence of a longitudinal bundle of fibres lying among the cells of the posterior division. Fusion between these two parts takes place in the upper part of the second sacral segment where the column inclines laterally, coming to lie between columns 2 and 3 anteriorly and column 6 posteriorly close to the lateral edge of the anterior horn. It disappears in the upper part of the third sacral segment in this position.

Column 6 appears in the lower part of the fifth lumbar segment posterior to columns 2 and 3 and situated at the lateral edge of the anterior horn. It increases in size throughout the first sacral segment and appears to be divisible into medial and lateral parts as it expands into the lateral white matter, causing a prominent bulge of the posterior part of the anterior horn. The lateral part which causes this expansion has been called column 7. These two columns, 6 and 7, are fused considerably throughout their length, and finally come together in the caudal part of the first sacral segment where their fused mass is large and is separated from columns 2 and 3 by the caudal part of column 5. With the loss of this latter column the two groups, formed from the fused columns 2 and 3 and the fused columns 6 and 7, come into contact; and, with the loss of the former in the third sacral segment, columns 6 and 7 become anterolateral in position and fade out just caudal to columns 2 and 3, except for a small part which is continued further caudally anterior to column 8 and disappears just before it.

Column 8 starts as a small mass of cells just posterior and slightly lateral to column 7 in the second sacral segment. It increases rapidly in size and is large throughout the third sacral segment, but is rapidly lost in the upper part of the fourth. It is the most posterior cell column of the lateral part of the anterior horn.

Thus it will be seen that there are eight cell columns in the lumbar enlargement and that their arrangement is in many ways similar to that found in the cervical enlargement. For example the most posterior column appears at the caudal extremity of each enlargement and extends further caudally than any other cell column in it. Also in each case there is a central column, column 5, which appears at the lateral edge of the anterior horn and, passing caudally, takes up a central position. The central column of the lumbar enlargement is much larger than that of the cervical region, and this is also true for all of the other columns of the lumbar enlargement with the exception of column 8, which is considerably smaller than its counterpart in the cervical region. It is possible that this difference in the size of the cell columns of the two enlargements (cf. Pl. 1, figs. 1, 2) is directly related to the amount of peripheral

mesoderm innervated by the cells in the columns. Again columns 3 and 4 of the cervical region are similar in position and relations to the columns 2 and 3 of the lumbar region, though the latter are more frequently fused than the former.

The general similarity between the arrangement of the cell columns of the two enlargements in mammals has already been noted by various workers, notably de Neef (1901), and it has been shown that similarly situated groups in the two enlargements supply analogous segments of the limbs (de Neef, 1901; Bok, 1928; etc.), the more anterior and cephalic cell columns supplying the proximal segments and the more posterior and caudal columns the distal segments of the limb (van Gehuchten & de Buck, 1898; van Gehuchten & Nelis, 1899; de Neef, 1901; Bok, 1928). During research on the development of these cell groupings the author has found them to be developed as a direct result of the connexion of the cells with the segments of the limb which they supply, thus suggesting that similar limbs are likely to have similarly arranged cell columns supplying them.

Van Gehuchten & Nelis (1899) described a posteriorly situated column in the lumbar enlargement as the nucleus of supply of the foot muscles. Since this is similar in position to column 8 it seems probable that the latter is functionally similar, and it is not surprising to find that this column is not so complex as its counterpart in the cervical region. This may probably be correlated with the small range of functional activity of the foot as compared with the hand.

On account of its position and its presence throughout the spinal cord, column 1 of both enlargements is probably a nucleus of supply of the trunk musculature, and it is especially large and divisible into two parts in the upper cervical region, so giving rise to column 2. The close relation of the latter to column 1 suggests a similar function, and its presence in the lower part of the third and throughout the fourth cervical segments makes it possible that column 2 is the phrenic column. This conclusion is borne out by Bruce (1901) who describes a similarly situated column as the nucleus of the phrenic nerve. Column 5 of the cervical region has been shown to supply the spinal accessory nerve and it extends to the caudal limit of this nerve, though Bruce (1901) states that the accessory nucleus ends in the fourth cervical segment.

When the columns described above are compared with the results obtained by other observers on adult material it is seen that there is considerable agreement as regards the number and position of the cell columns present. Though the classical work of Bruce (1901) does not name as many columns as are described here, he admits the subdivision of several of his columns to make up a like number. Very similar results have been obtained by Jacobsohn (1908) for the cervical and lumbar regions and by van Gehuchten & de Neef (1900) for the lumbar region only. Thus it seems probable that as early as the fourteenth week of foetal life the cell columns have attained an adult arrangement.

SUMMARY

The cell columns in the cervical and lumbar enlargements of a human foetus are described and compared and several points of similarity are shown.

The cell columns are already adult in their arrangement by the fourteenth week of foetal life.

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EXPLANATION OF PLATE 1

Fig. 1. Transverse section through the fifth cervical segment of the spinal cord of a human foetus of fourteen weeks. Stain, toluidin blue. ×70.

Fig. 2. Transverse section through the second sacral segment of the spinal cord of a human foetus of fourteen weeks. Stain, toluidin blue. ×70.

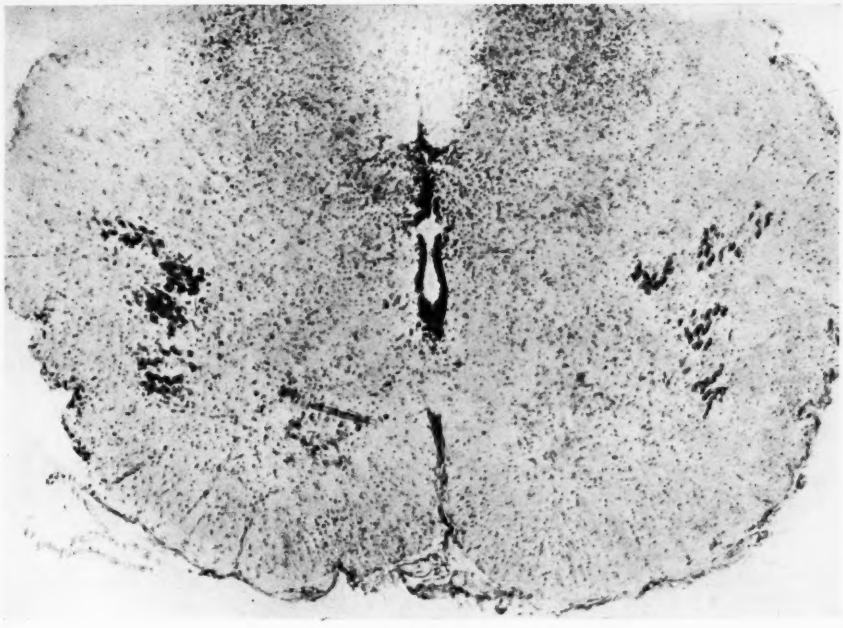

Fig. 1.

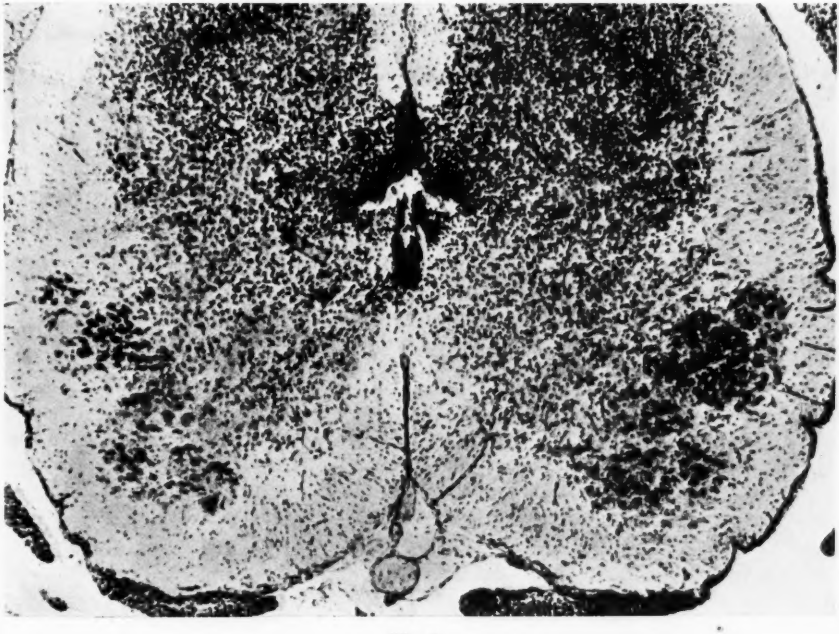

Fig. 2.

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THE CHORION AND ENDOMETRIUM OF THE EMBRYO H.R. 1

By T. B. JOHNSTON
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In the preceding number of this *Journal* a detailed description was given of the Embryo H.R. 1; the present paper deals with the chorion and the endometrium of this embryo.

THE CHORION

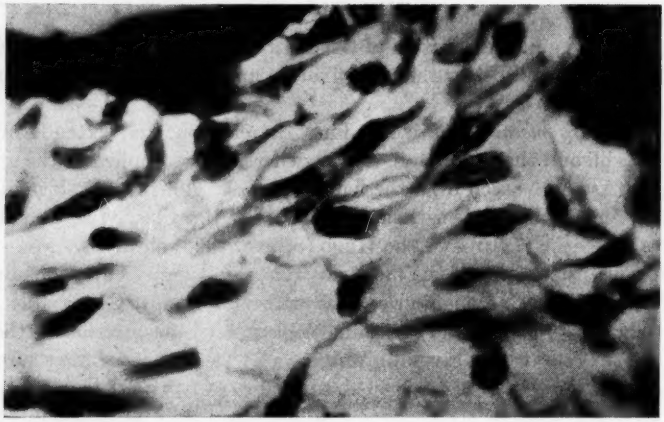
The fixation and preservation of the constituent parts of the chorion were, not unnaturally, still better than the fixation and preservation of the embryo itself.

The *primary mesenchyme* which lines the chorion, and extends into the chorionic villi and their branches, is everywhere closely applied to the cytotrophoblast. As will be seen from the figures already published no shrinkage has occurred, and in this respect the tissues are as well preserved as they were in the Strahl-Beneke (1910) and the Hugo (Stieve, 1926) embryos.

The constituent cells of the mesenchyme exhibit many variations in form, but the majority are elongated and lie with their long axes parallel to the cytotrophoblast both in the chorionic wall and in the interior of the villi. (1) Fusiform cells with branching processes at each end are very numerous (Text-fig. 1). They tend to occur in short strings of two or three, especially in the villi. Their nuclear structure is distinct; the nucleolus is prominent and the chromatin network is clearly shown, although it is not darkly stained. (2) Elongated, rod-like forms also are numerous. Their nuclei stain so darkly that their structure cannot be made out. These cells also tend to occur in short strings which suggest the angioblastic strands described by MacIntyre. (3) Smaller, rounded, cells with deeply staining nuclei and scanty cytoplasm form a less numerous group. (4) In addition, short rod-like forms occur with deeply stained nuclei. They may be the earlier stages of group (2), or they may be cells of group (2) which have been cut obliquely to their long axes. (5) Within the villi, and in places in the chorionic wall, large mesoblasts with oval nuclei and branching protoplasmic processes are common. Their nuclear structure is clearly shown and they appear to represent early stages of the cells in group (1).

No indubitably vascular spaces and no blood-islands are present in any part of the chorion, although angioblastic strands, of rather doubtful identity, were recognized in the apical region of the chorion. Hertig (1935) has recently put forward the view that both mesoblasts and angioblasts are direct derivatives of the cells of the cytotrophoblast and claims to have recognized angio-

blastic strands in the mesenchyme of the Miller ovum. If his interpretation is correct, it is strange that no obvious angioblastic tissue can be identified in the chorionic mesenchyme of H.R. 1 and that such as is present has not developed in the interval between the two stages, for the H.R. 1 is certainly at least two and not improbably three or even four days older than the Miller ovum. At the same time it must be remembered that H.R. 1 required to be reblocked after the chorionic cavity had been opened and it is possible that in the process the superficial parts of the chorionic mesenchyme might have suffered injury or loss. In this way the absence of the mesothelial layer, which MacIntyre (1926) has described as present in embryo T.B. 2, may be accounted for, because it is only in a few places that cells are present which are worthy of the name "mesothelial".



Text-fig. 1. Primary mesenchyme of a chorionic villus, showing numerous fusiform and branching cells. × 950.

The *Chorionic Villi* cover the whole of the outer surface of the chorion. They are longest and most crowded together in the region of the decidua basalis, where they average 0·6 mm. in length. In this situation they show fewer branches than in the lateral walls of the chorion, where they tend to be both broader and shorter. Being more widely spaced they are able to branch freely and as many as 10–12 branches may arise from one villus. In the region of the decidua capsularis the villi are shortest and measure on the average 0·2 mm. They are narrower than the villi in the lateral walls and show fewer branches.

The cells of the mesodermal cores of the villi are specially well preserved and show numerous branching and anastomosing processes. Fusiform cells predominate and arrange themselves in the long axis of the villus or its branches.

The *Cytotrophoblast* forms an easily recognizable layer over the whole surface of the chorion and the chorionic villi. The cells are regularly arranged

and show little variation in size. It is a curious fact that although most of them possess nuclei with a clearly defined chromatin network, many of them exhibit darkly staining pycnotic nuclei in which no trace of structure can be discerned.

From the apices of the villi typical cell columns are continued into the trophoblast shell which forms the outer boundary of the intervillous space. The cells in these columns vary considerably in size. The smaller cells are found at the apices of the villi, where they are closely crowded together. The larger cells are found in the trophoblast shell and show large intracellular shrinkage spaces (Pl. 1, fig. 1). In addition the chromatin network of the nuclei is irregularly disposed and appears to have undergone shrinkage in many places, leaving clear areas which are unstained. The appearance indicates that many of these cells are undergoing degenerative changes. It is certain that at the apices of the villi the cell columns are actively growing and it is a curious fact that in nearly 400 sections only a few, very doubtful, mitotic figures were seen. Florian (1928) has suggested that the nuclei in the plasmodium increase by amitosis, and the absence of mitotic figures strongly suggests that the same process is the normal method of cell division in the cytotrophoblast.

The plasmodial trophoblast. The cytotrophoblast of the chorionic membrane and of the chorionic villi is everywhere covered with a thin layer of *resorption plasmodium* (Florian, 1928). It is this layer which is bathed with the maternal blood in the intervillous space and through which all the nutritive substances must pass before they can reach the embryo. It has the appearance of a very fine foam, and its free surface shows the well-recognized "brush border". The contained nuclei are irregularly spaced, and tend to be flattened against the cytotrophoblast. For the most part they are darkly staining and their structure is difficult to discern. No mitotic figures were seen, but, on the other hand, no dumb-bell forms, such as Florian has described, were encountered.

The cytotrophoblast of the cell columns is covered for the most part with plasmodium, but there are many patches where no covering plasmodium is visible and the chorionic surface of the cytotrophoblast shell is in direct contact with the maternal blood of the intervillous space.

Islands of free plasmodium are found in the intervillous space. They are usually pale in colour; the fine foam is coarser in character and contains numerous vacuoles; and the contained nuclei show obvious retrogressive changes. In many of these islands the nuclei are dark and pycnotic and have lost their regular outline; in others the nuclei are swollen and hydropic, are pale in colour, and contain only a nucleolus and some shrunken shreds of chromatin. They are obviously in process of dissolution. Although they occur generally throughout the intervillous space, they are few in number under the decidua basalis and numerous under the decidua capsularis.

Areas of healthy looking plasmodium occur in the midst of the trophoblast shell and around the periphery of the shell in contact with the endometrium. Many of these areas line slit-like spaces in the shell (Pl. 1, fig. 3), but others appear to be surrounded completely by the cytotrophoblast. Where a free

surface presents itself for examination, the "brush border", which is so characteristic of the "resorption plasmodium", is absent or inconspicuous. Florian (1928) has termed this the "proliferative plasmodium", as he believes that it differs materially in its function from the "resorptive plasmodium".

Both in the trophoblast shell and in the peripheral zone the strands of proliferative plasmodium are easily recognized on account of their distinctive staining reactions. The nuclei stain so darkly with haematoxylin that their structure can be made out only with difficulty, while the surrounding plasmodium stains reddish pink with eosin. In the peripheral zone, particularly, the nuclei are elongated, being fusiform or rod-like, and the associated plasmodium is drawn out into strands in the long axis of the nucleus. Some of these strands contain three or four nuclei end to end and appear to be actively streaming into the endometrium (Pl. 1, fig. 1). In the maternal tissues they are found at considerable distances from the trophoblast shell. In these situations it is exceedingly difficult to convince oneself that they form part of an open-meshed plasmodial network and are not independent units. They can be seen in contact with the walls of the maternal blood vessels and uterine glands, and the adjoining maternal cells present the appearances of incipient degenerative changes. Occasionally the nuclei of these plasmodial strands are V- or Y-shaped, and in places they lose their elongated shape and become irregularly quadrangular. In the latter case the surrounding plasmodium adapts itself to the altered shape of the nucleus. In many situations one strip of plasmodium contains two elongated nuclei lying side by side, and these forms, together with the V- and Y-shaped nuclei, give support to Florian's view that in the plasmodial trophoblast amitotic nuclear division is the rule. The Y-shaped nuclei represent the first stage, the V-shaped nuclei the second, and the side-by-side nuclei the third and completed phase of the process.

The proliferative plasmodium can be seen in close relationship with the uterine vessels, which it is presumably attacking. The walls of the arterioles concerned are thickened and their lining endothelial cells are swollen. In many places the proliferative plasmodium has replaced the endothelium lining the wide venous sinuses which surround the ovum, and in these situations its margin is always clear cut and no "brush borders" are apparent.

Within the trophoblastic shell there are numerous straight channels which are parallel to the veins in the adjoining part of the stratum spongiosum. They are lined for the most part with the proliferative plasmodium (Pl. 1, fig. 3), but here and there small patches retain a lining of endothelium. These spaces and the presence of proliferative plasmodium in the walls of the venous sinuses immediately outside the trophoblastic shell suggest that, after the implantation of the ovum, the implantation cavity is enlarged, in part at least, by the incorporation of the adjoining venous sinuses. The mechanism of the process appears to be as follows. Strands of proliferative plasmodium attack the walls of the vessel, destroy and replace the lining endothelium. The trophoblastic shell enlarges at the expense of the intervening maternal tissues until the sinus

becomes separated from the intervillous space only by the proliferative plasmodium and the trophoblastic shell. Degenerative changes occur in the trophoblast so that new spaces are formed which enable the sinus to communicate with the intervillous space. A new trophoblastic shell is then formed on the opposite side of the sinus, which gradually loses its identity, becoming merged eventually in the intervillous space.

Text-fig. 2. The apical part of the triangular chorionic cavity and the adjoining part of the stratum spongiosum, showing the absence of the "saw-teeth" appearance in the uterine glands in the immediate vicinity of the ovum. ×30.

THE ENDOMETRIUM

The uterine mucosa presents the typical appearances associated with an early pregnancy. The compact and spongy layers are quite characteristic.

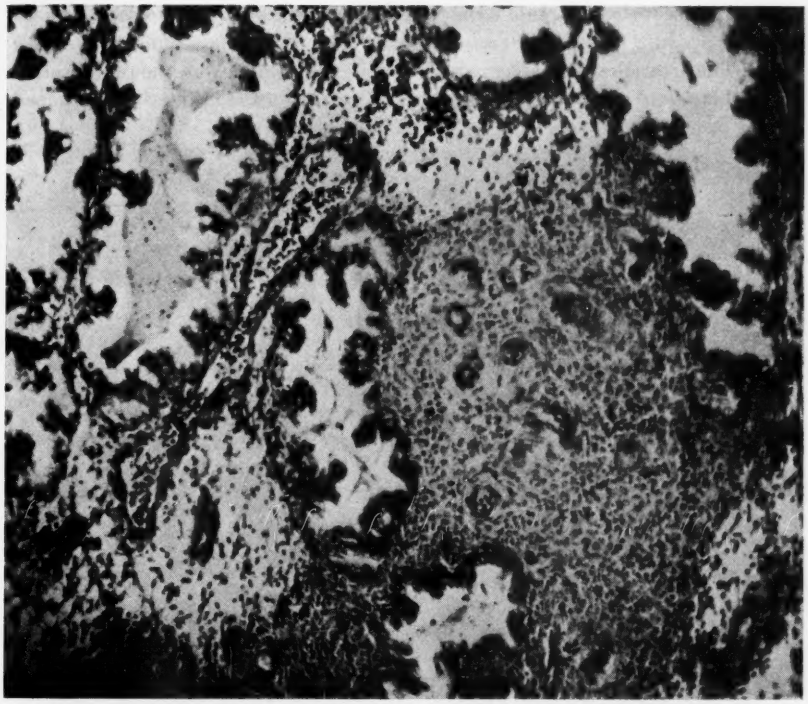
The glands of the stratum spongiosum are enormously dilated and full of secretion, and in the vicinity of the ovum some contain extravasated blood. The lining epithelium displays the "saw-teeth" appearance, except in the immediate neighbourhood of the ovum, where the walls of the glands are much less irregular than they are elsewhere (Text-fig. 2). Presumably these glands

have been subjected to considerable internal pressure, for their lining epithelium is flattened in many places and in some situations has disappeared.

The functional significance of this excessive glandular enlargement has been discussed recently by Falkiner (1932). Like many other writers he accepts the view that the secretion of the uterine glands provides nourishment for the ovum after it reaches the uterine cavity and prior to implantation. It is, however, a commonplace that in tubal and ovarian gestations the ovum may develop and form a normal embryo, although it is then unable to benefit by the secretion of the uterine glands. Again, the degree of dilation and the amount of the secretion are out of all proportion to the amount of nourishment required by the ovum. It is clear therefore that the production of "embryotrophe" is not only not the principal function of the glandular activity but that it is very doubtful indeed whether the ovum derives any nourishment from this source. In addition to the function of producing "embryotrophe", Falkiner ascribes to the glandular enlargement certain mechanical functions. He believes that it exerts "a definite effect on the blood supply at the commencement of pregnancy, favouring the formation of a venous sinus". With this view the appearances in the stratum spongiosum of H.R. 1 are in complete agreement. The compression exerted by the enlarged glands in the immediate vicinity of the ovum on the venous return from the area is seen in a large number of the sections, and undoubtedly helps to determine the character of the circulation in the intervillous space, which will be considered later in this section. In addition, Falkiner ascribes to the glandular enlargement the function (1) of forming a layer impervious to the action of the trophoblast, (2) of providing a natural line of cleavage, and (3) of providing a layer from which the mucous membrane may be regenerated *post partum*. That the glandular epithelium is very resistant to the destructive action of the proliferative trophoblast can be inferred from the appearances in numerous sections of H.R. 1. In many situations, ducts, surrounded by a small amount of stroma, are almost isolated by the trophoblast from the rest of the endometrium. Where "peninsulas" of the mucosa occur, they almost invariably contain ducts.

The stratum compactum is infiltrated with a large number of small round cells which are obviously lymphocytes. No typical decidual cells are present. Small areas of localized oedema, which tend to spread into the more superficial parts of the stratum spongiosum, are a prominent feature. In many situations these areas are traversed by small veins which contain a disproportionately large number of white blood corpuscles (Text-fig. 3). A similar condition has been noted in the endometrium by Stieve, Falkiner and many other observers. Sandison (1932) has shown that white blood corpuscles may occur in unusually large numbers in "any uncontracted blood vessel in which the circulation has temporarily ceased, but which remains connected with other circulating vessels". This phenomenon is therefore associated with extremely sluggish circulation, and attention must be drawn to the evidence of such a type of circulation in the immediate neighbourhood of the ovum.

Large, thin-walled, venous sinuses are found in all young human ova closely adjoining the implantation cavity. They communicate freely with the intervillous space; they communicate with one another; and they are drained by a number of very large veins. One of these sinuses is shown in Pl. 1, fig. 2. It is

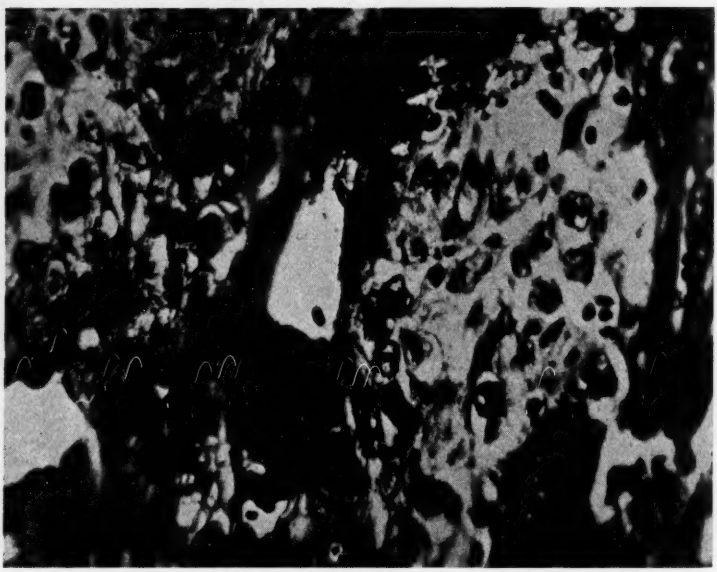


Text-fig. 3. The deepest part of the stratum compactum in the neighbourhood of the ovum. In the left half of the figure a vein is shown cut obliquely as it lies between two uterine glands. Note the patch of oedema which surrounds its upper and lower ends. In the right half of the figure one of the tortuous arterioles has been cut in several places. The stroma by which it is surrounded is non-oedematous. × 100.

placed deep to the decidua basalis and three veins are shown leading from it into the deeper layers of the stratum spongiosum. Falkiner (1932) has suggested that these sinuses owe their origin to the pressure of the dilated glands on the neighbouring veins, and the appearances in the embryo H.R. 1 certainly support his view.

If we now turn to a consideration of the arteries in the stratum compactum, we find a very different picture. Barthelmez (1931) and Daron (1936) have shown that, apart from some minute branches which are restricted to the deepest part of the stratum spongiosum, all the arteries follow a very tortuous

course, so that the same vessel is cut in many places in the same section. This character makes the arteries easy to identify but difficult and tedious to trace. They run in the interglandular tissue surrounded with a sheath of stroma, whereas the veins run in close relationship to the walls of the glands and have little or no surrounding stroma in the stratum spongiosum. In the vicinity of the trophoblast shell in H.R. 1 the tissue around the smaller arteries shows no signs of oedema (Text-fig. 3) as compared with the tissue around the smaller veins. The arterioles themselves are the subject of attack from the proliferative trophoblast and show resulting changes. Their walls are noticeably thickened



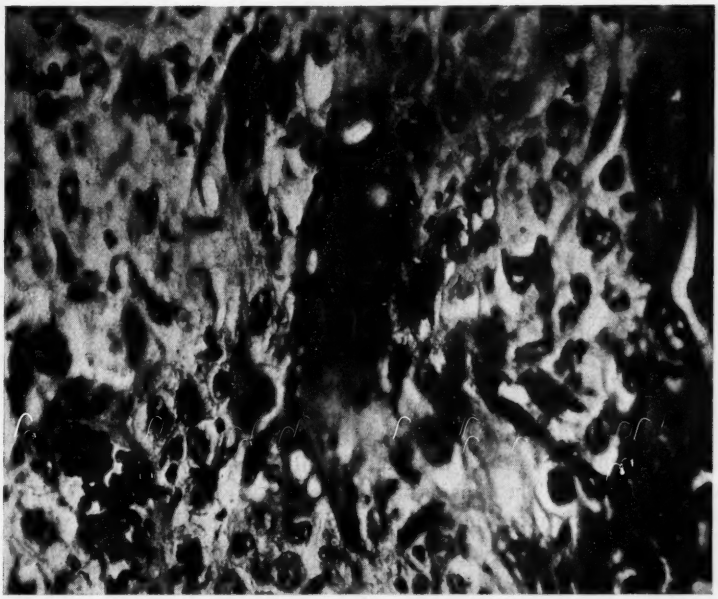
Text-fig. 4. A small arteriole in the stratum compactum close to the trophoblast shell, which lies to the right in the figure. The lumen of the vessel is partially occluded by clotted blood and the walls of the patent portion show extensive fibrinous changes. × 960.

and the thickening involves both the endothelial lining and the outer walls. Despite a very prolonged search I was unable to trace any of these arterioles into the intervillous space or into any of the venous sinuses. In many situations small branches of the arteries almost succeeded in reaching the intervillous space but their ends were always occluded and usually sealed off by fibrin. A similar fibrinous change was found in several of the larger arterioles in the stratum compactum in the immediate neighbourhood of the ovum (Text-figs. 4, 5).

Although no arterioles were found opening into the intervillous space or the adjoining venous sinuses, a number of dilated capillaries were found in the

decidua capsularis which undoubtedly established communications either directly or indirectly with the intervillous space. These capillaries contain the normal proportion of white to red cells and their walls are not conspicuously thickened.

Falkiner succeeded in tracing one arteriole into one of the sinuses, and Stieve (1926) has figured a vessel, which he identifies as a small artery, opening indirectly into intervillous space. He states further "Die Einmündung der



Text-fig. 5. The same arteriole as in Text-fig. 4, five sections (25 μ) later. It is now completely occluded and sealed off with fibrin. × 960.

Arterien in die weiteren Bluträume lässt sich an mehreren Stellen gut beobachten". Nevertheless, he concludes that the circulation in the intervillous space and in the sinuses is exceedingly slow.

In H.R. 1 the blood in the intervillous space and the sinuses must have been almost stagnant. Only a relatively small number of capillaries could be traced into the space so that the inlet must have been negligible. The sealing off of the small branches of the arteries seems to be a precaution to prevent a rapid circulation at this stage. Indeed, the areas of patchy oedema, the disproportionate number of leucocytes in the superficial and many of the deeper veins, the size of the venous sinuses, the sealing off of the arteries, and the capillary type of inlet, all point in the same direction. They indicate that,

prior to the formation of blood vessels in the chorionic villi, and the establishment of the chorionic circulation, efficient nutrition of the embryo can only be ensured provided that the circulation in the intervillous space is so slow that the contained blood is practically stagnant.

One must not, however, overlook the fact that far more than adequate provision is made for the venous drainage of the sinuses and the intervillous space. The presence of so many large and intercommunicating veins leading away from the immediate neighbourhood of the ovum suggests that the establishment of free circulation in the intervillous space at a later stage is not a gradual but rather a comparatively sudden process and ensures that increased entry of blood into the space, no matter how extensive, will not overtax the carrying capacity of the outlets.

The point of entry of the ovum was not identified. A large part of the decidua capsularis is covered with a substantial and comparatively recent blood-clot. Where it is exposed on the surface it is covered with cubical epithelium, which, however, is not in a very good state of preservation. In its central part, where the point of entry probably was situated, a sheet of fibrinoid substance, staining well with eosin, forms nearly the whole thickness of the decidua. Its deep surface is covered, for the most part, with plasmodial trophoblast, but some shrinkage has apparently occurred in this region and has resulted in the separation of the trophoblast from the decidua. This sheet of fibrinoid substance is penetrated by strands of proliferative trophoblast and here and there contains broken-down cells which are apparently duct epithelium and stroma cells. In the peripheral part of the decidua capsularis the stroma cells become more numerous but are still intermingled with strands of proliferative trophoblast. The remains of ducts are easily recognizable and numerous dilated capillaries are present. The endothelial cells of these small vessels are slightly swollen and they are replaced in many situations by single units of the trophoblast. They contain a normal proportion of red blood corpuscles and many of them open into the venous sinuses or the intervillous space. In this part of the decidua capsularis small groups of lymphocytes are frequently to be observed.

In the peripheral zone it is not, as a rule, difficult to differentiate the foetal from the maternal tissues, although there is nowhere any necrotic zone such as is present in earlier stages, e.g. T.B. 1 (Bryce, 1908) and von Möllendorff's Sch. (1921). Here and there fibrinoid patches are encountered and the maternal cells for the most part show signs of degenerative changes. The ducts appear to be least affected by the destructive process that is going on all around them and in many places are almost isolated by the trophoblast.

SUMMARY

1. Evidence is advanced in support of the view that the implantation cavity is enlarged by the incorporation within it of some of the large venous sinuses which are found in close proximity to the ovum.

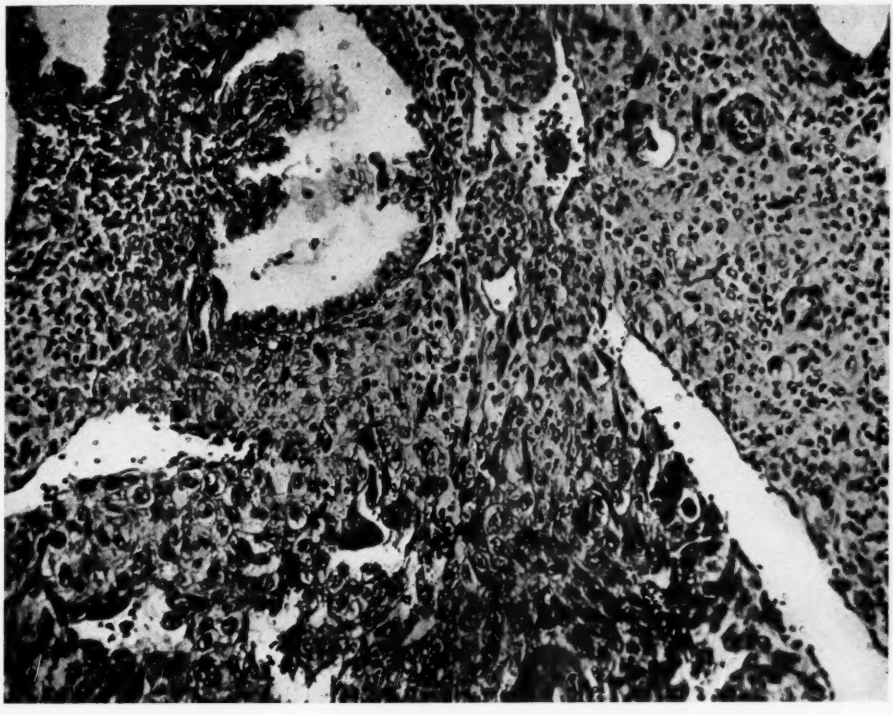


Fig. 1.

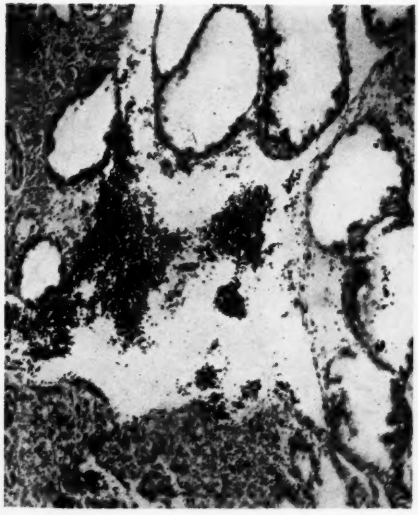


Fig. 2.

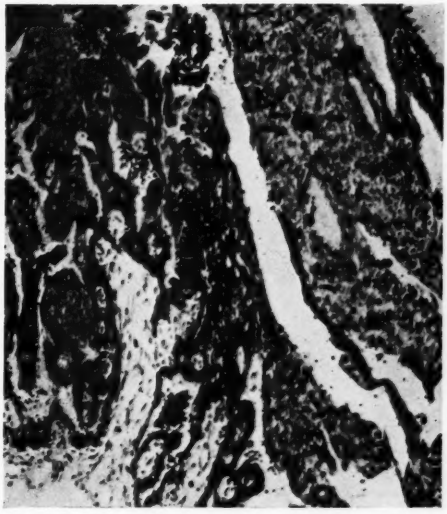


Fig. 3.

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2. Proliferative plasmodial trophoblast can be distinguished from the resorptive plasmodium by the absence of a "brush-border" where it presents a free surface or edge.

3. It is suggested that there is practically no circulation in the intervillous space at this stage. Evidence is advanced to show that as the walls of the arterioles in the endometrium are destroyed, the vessels are obliterated and do not open into the intervillous space, which is dependent for its inflow on capillaries in the decidua capsularis.

4. The presence of a great number of large calibre veins leading away from the venous sinuses, and so from the intervillous space, is regarded as a provision to ensure a ready outflow from the space when free circulation is established through it.

I wish to express my indebtedness to the University of Edinburgh for permission to publish this paper, the substance of which formed part of a thesis submitted for the degree of M.D. I am also indebted to Mr G. A. Walker, Senior Technical Assistant in the Anatomy Department, Guy's Hospital Medical School, who was responsible for the microphotographic work.

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EXPLANATION OF PLATE 1

Fig. 1. The trophoblast shell adjoining the apex of the triangular chorionic cavity is shown in the lower part of the figure. It includes the larger cells from the apices of the cell columns and amongst them some patches of proliferative plasmodium can be seen. Strands of proliferative plasmodium are streaming into the adjoining endometrium and can be recognized by the elongated shape and dark staining of their apparently isolated units. In the upper right-hand part of the figure apparently discrete units have penetrated deeply into the endometrium. ×158.

Fig. 2. A dilated venous sinus, lying deep to the decidua basalis. Three large veins leave its upper (in the figure) border. ×52.

Fig. 3. One of the slit-like spaces in the trophoblast shell, partially lined by proliferative plasmodium. One of the large basal villi is shown in the lower left quadrant of the figure, cut obliquely in its long axis. ×80.

THE CUTANEOUS LIGAMENTS OF THE DIGITS

By J. GRAYSON
University of Manchester

The so-called cutaneous ligaments of the human digits were first described by Prof. John Cleland in 1867. These ligaments constitute a distinct fibrous septum dorsal to the digital nerves and vessels. Their function is presumably that postulated by Cleland of retaining the skin at the sides of the joints and thus preventing "bagging" during movements of the joint. The ligaments arise from the sides of the phalanges and are inserted into the skin over the interphalangeal joints. In a series of dissections undertaken by the writer it has been demonstrated that these ligaments are present not only in the human adult and in the human foetus, but also in a lemur (*L. varius*), in the Celebes Black Ape (*Cynopithecus niger*) and in more generalized mammalian forms. Cleland's description of the human condition was verified in every case, except that in *Lemur* the ligaments are somewhat thin and diffuse, forming parts of a continuous membrane rather than a series of discrete individual structures. In other animals dissected, and especially in adult man, they are relatively strong, well defined and discrete, and clearly capable of functioning as skin ligaments, acting from their fixed attachment to the bone.

But in addition to these structures it was apparent that there is also a fibrous septum volar to the digital nerves and vessels. This septum forms a series of retinacula which, if we refer to Cleland's ligaments as the deep digital skin retinacula, might well be termed the superficial or volar retinacula. Their distribution is such that they could obviously subserve precisely the same function as their deeper fellows (see Fig. 1).

The volar skin retinacula arise, not from the phalanges themselves, but from the flexor sheaths, and they are associated in pairs with each interphalangeal joint. Those associated with the proximal interphalangeal joint consist of two pairs of ligaments. The proximal members arise from the flexor sheath over the distal third of the proximal phalanx, and the distal pair from the sheath over the proximal third of the intermediate phalanx. These ligaments pass respectively in a distal and proximal direction as they go to be inserted into the skin over the sides of the proximal interphalangeal joint. In dissections of the distal interphalangeal joint only the proximal pair of ligaments could be demonstrated with certainty. It is of interest to note that Cleland described a deep ligament of the distal joint: but this is not shown in the figure accompanying his paper, nor was its presence satisfactorily demonstrated in the present investigation.

It seems obvious, from the examination of the types dissected here, that the strength of the volar ligaments is in inverse proportion to that of their

deep fellows. Thus in *Lemur* the superficial ligaments are by far the thicker and are clearly the only structures that can act as skin retinacula. *Cynopithecus* and the human foetus showed the volar ligaments developed to about the same degree; but in *Cynopithecus* the dorsal retinacula, although strong, are not well differentiated as separate entities. In the adult human digit the volar ligaments are thin and membranous, almost as though they had undergone passive stretching in the growth of the digit from the foetal condition. It was noted that the volar retinacula occupy the same plane as the ligamenta natatoria of Grapow (bandelette transversale sous-cutanée of Bourgery or interdigital ligaments of Legueu and of Juvara) and the superficial transverse ligament, both probably derivatives of the primitive interdigital web. It seems, therefore, not unlikely that the volar retinacula are derived from the same fibrous layer. In order to test this hypothesis, the manus of *Ornithorhynchus* was dissected and it was found that in its web there are two distinct membranous layers between which the digital vessels and nerves run. The volar of these two membranes passes from flexor sheath to flexor sheath of adjacent digits, whereas the dorsal membrane arises from the phalanges and occupies the intervening interphalangeal spaces. This double web membrane may well be the basis from which both superficial transverse ligaments, the ligamenta natatoria, and both sets of digital skin ligaments are derived. Reacting to the stresses imposed by continuous flexion, these two membranous planes would appear to give rise to a dual series of skin retinacula variably differentiated according to the varying demands of specialized digital mobility.

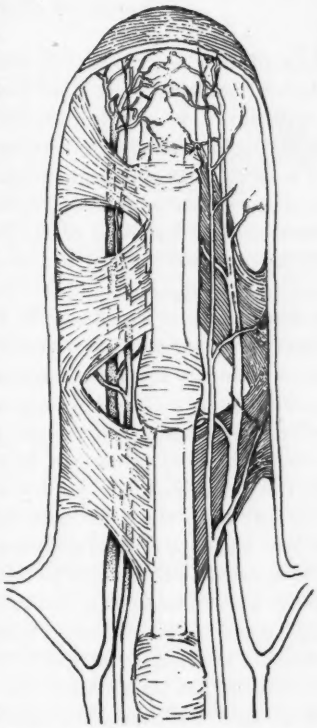

Fig. 1. A composite drawing, made from drawings of a number of dissections, showing the cutaneous ligaments of the digits from the volar aspect. The dorsal ligaments are seen on the right side of the figure, and the volar ligaments on the left.

I have to thank Prof. Wood Jones for the figure which is a composite made from my own drawings of the dissections carried out during the investigation.

REFERENCE

CLELAND, J. (1878). *J. Anat. Phys.* **12**, 526, Pl. XVII. (Cleland first reported the existence of these ligaments at the Meeting of the British Association in Dundee in 1867.)

THE MECHANICAL ANATOMY OF THE CARPUS AND ITS BEARINGS ON SOME SURGICAL PROBLEMS

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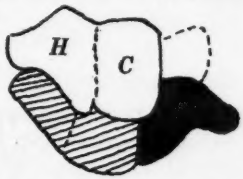
Up till now descriptions of the working of the wrist have been kinematical rather than functional. They have been concerned with the motions of the two carpal rows, or of the individual bones, as geometrical phenomena: to be referred, for example, to certain axes of rotation, rather than as the workings of a set of parts of a single mechanism, the understanding of which would lead to a more rational opinion regarding the causes of traumatic defects and to a more critical handling of their treatment and after-care. The discovery of X-rays in 1895 opened a new path to the few who were interested in the workings of the joints; and as early as 1896 studies upon the wrist-joint were published from the Glasgow University anatomical school by Cleland & Mackay (1896) and by Bryce (1896). In 1911 Fick published the last of his three volumes upon the anatomy and workings of the joints: these have a special interest, for they appear to form the basis of whatever details are offered upon joint movements in to-day's standard text-books of anatomy, whether these are American, British or Continental. Fick's work was kinematic, as this term has been defined above; his own investigations were thorough, whether upon the cadaver or upon the living; and his review of the work of others was both critical and encyclopaedic, save for one omission: he neglected the contribution of John Goodsir (1858), who had insisted that the synovial joints (diarthroses) were not only places at which bones could be moved, but also places where they could be more tightly, and even rigidly, bound together, should the economy of the body demand it. Four years later Bryce (1915) remarked that Fick's book had displaced all earlier works on the subject: I would add that practically all subsequent investigators of the wrist have only followed and confirmed Fick's statements and observations, that is, within the limits he set for himself. These later investigators, however, have not always been aware of how much Fick had done and had published. For example, one excellent paper (by Wright, 1935) refers to a paper of Fick's published in 1901, having no reference to his later, more detailed, publication. Wright goes on to describe his own results, which are essentially those of Fick, and may therefore be taken to be confirmatory of them. So, also, the papers of Cyriax (1926) and of von Bonin (1929) cover ground already explored and described by Fick, on secondary movements and passive rotation respectively; and their conclusions agree with his. It appears, then, that there is a very complete body of knowledge about the individual carpal bones and their movements: so that this knowledge can be taken and used as a basis for further inquiry into the working of the carpus as a unitary structure.

A FUNCTIONAL SUBDIVISION OF THE CARPUS

The trapezium (multangulum majus) is to be excluded from our consideration, and the pisiform will be looked on as part of the apparatus by which the wrist is moved: the carpus can thus be defined for the present as consisting of all those bones which lie between the forearm and the metacarpals II–IV inclusive. The carpus so defined is divisible into three masses: the navicular (scaphoid) bone; the lunate and triquetral bones together; and a distal mass formed of the hamate, capitate and trapezoid (lesser multangular) conjointly. The essence of this division is the separation of the navicular from the other bones of the proximal carpal row: it is based upon the observed fact that this bone moves at times with the proximal and at times with the distal row and it appears to have first been explicitly made by von Bonin, although it is implicit in Fick's descriptions. The carpus defined and divided as stated is shown in Text-fig. 1.



Text-fig. 1. The carpal bones considered in the present study. Hamate and capitate: simple outline. Triquetral and lunate: striped. Navicular: solid black. Triquetrum: dotted outline.

THE CARPAL GRADIENTS OF ANGULAR VELOCITY

There are two synovial cavities between the forearm and the distal carpals, that is, between the origin and the insertion of any muscle producing movement of the hand upon the forearm or conversely. Volar flexion and dorsiflexion, radial deviation and ulnar deviation are all angular displacements of the forearm and the proximal row about the head of the capitate, even although the forearm be the fixed part and the hand the moving part: this simple application of Newtonian relativity greatly simplifies the analysis of radiographs of the living wrist. The contraction of, for example, the mm. extensores carpi produces an angular velocity of the forearm bones relative to the capitate, and also an angular velocity of the proximal row relative to the same bone. Text-fig. 2 is composed of superimposed tracings of radiographs of the wrist-joint in full volar flexion and in full dorsal flexion: the tracings give the capitate bone the same orientation in each posture. The figure shows that the lunate slides round the capitate and that the radius slides round the lunate to an even greater degree: for the radius (in dorsal flexion) starts from a more volar position relative to the capitate than does the lunate, and ends in a more dorsal position relative to the same bone. The angular velocity of the radius is, then, greater than the angular velocity of the lunate in movements about an axis passing from the medial to the lateral side of the wrist: there is a proximo-distal gradient of angular velocity across the carpus, and it operates in dorsal and in volar flexion. This statement assumes, incidentally, that movement between the capitate and lunate on the one hand, and between the lunate and

radius on the other, continues throughout the period of displacement. The assumption is true: both Fick (1911) and Wright (1935) draw special attention to the fact, and my own observations confirm it. The velocity gradient arises from the "slip" between the bones of the proximal row and their proximal and distal neighbours, and the slip is due to the presence of the synovial fluid. I reserve any fuller discussion of the origin of the velocity gradient for a later paper which will deal with some aspects of the general physics of the joints.

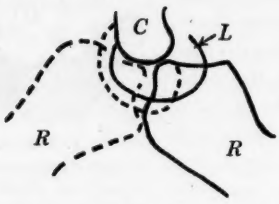


Fig. 2.

Fig. 3.

Text-fig. 2. Displacements of the lunate and radius on the capitate in dorsiflexion of the wrist. Tracings of radiographs in full volar flexion (full line) and in full dorsiflexion (broken line), photographed from the radial side, to show that the angular velocity of the radius is greater than that of the lunate.

Text-fig. 3. Rotation of the navicular bone in dorsiflexion of the wrist, the capitate being taken as fixed. The upper figure shows the tubercle of the navicular in full volar flexion (full line) and in extension (*Normalstellung*, broken line). The lower figure shows the tubercle and the lunate bone in extension (full line) and full dorsiflexion (broken line). The navicular moves with the first row in the first phase of dorsiflexion, and with the second row in the second phase. (First row = proximal row.)

THE ROLE OF THE NAVICULAR BONE

The continuous operation of the velocity gradient applies to that part of the carpus which lies medial (ulnar) to the navicular bone. The navicular, on the other hand, plays a special part in the carpal mechanism on account of the fact that it articulates with both the proximal and the distal carpal masses. Fick drew attention to the fact that this bone moved with the proximal row in the earlier stage of dorsiflexion, and with the distal row in the later stage. Conversely, the navicular moves with the distal row in the earlier stage of volar flexion, and with the proximal row in the later stage. The transition between earlier and later stages in these movements is marked by the so-called *Normalstellung*, that is, by the position in which the hand is outstretched in line with the forearm. I have repeated Fick's observations radiographically (Text-fig. 3); and have observed the same phenomenon upon the dissected wrist, as have also several of my colleagues. In the course of the movement from full volar flexion to full dorsal flexion there is, therefore, movement of the capitate upon the navicular up to the position of extension (i.e. *Normalstellung*); thereafter these bones are at rest upon each other but move together upon the lunate until

full dorsiflexion has been attained. Conversely, in the movement from dorsiflexion to full volar flexion the capitate and navicular move upon the lunate up to the posture of extension of the hand; thereafter the capitate moves upon the lunate and navicular which are, in their turn, relatively at rest. These changing dynamical relations of the navicular bone provide the key to the mechanism of the carpus.

THE CARPUS AS A SCREW-VICE OR CLAMP

The carpus, as it has been defined and illustrated at the beginning of this paper, can now be stated to consist of two rows, each row having three bones in it, and one bone (the navicular) being common to the two rows (Text-fig. 1). The trapezoid is neglected in this connexion. The medial or ulnar ends of the two rows are connected by their common attachment to a conical mass of fibres which is part of the deep portion of the internal lateral (ulnar collateral) ligament of the wrist; their lateral or radial ends are connected by their common attachment to the navicular. Consideration may now be given to the movement of dorsiflexion of the hand, the forearm being fixed.

The muscular forces acting upon the metacarpal bones directly are transferred practically undiminished to the hamate, capitate and trapezoid, and these three bones rotate about an axis which passes through the head of the capitate and the glenoid fossa of the navicular with which the capitate articulates. The movement between these two bones is a twisting movement, and one which tends, therefore, to bring them into close contact: for the ligaments which connect them are not elastic and so draw the apposed surfaces together until they are at rest upon one another and are held so under pressure. When this state has been attained—and it has been seen to be attained in the position of extension of the hand—the navicular bone must follow the movement of the capitate as it passes further into dorsiflexion, and the small crescentic facet which it bears for articulation with the lunate will glide upon that bone.

The hamate rotates with the capitate. "The articular facet on the unciform (hamate) shows a groove starting from below, winding in a spiral fashion and terminating posteriorly; it seems as if the bone had been caught at its apex and twisted" (Johnston, 1907). The photograph in Text-fig. 4 will make Johnston's description clear; and it will also make clear my further statement that the proximal articular surfaces of the hamate and the capitate bones form, together, a very evident screw surface: or, at least, as much of one as could be brought into action in the less than fully circular compass of the movements of the wrist. This screw surface is a male surface: the female surface (*Mutterschraube*) is formed by the corresponding articular facets on the cuneiform (triquetrum, pyramidale) and the lunate bones. A special dissection is advisable to see this carpal screw *in situ* in the most effective way.

An upper limb is completely cut across at the junction of the upper two-

thirds of the forearm with the lower third. The lower piece of the forearm and the hand are next partly divided into a dorsal and a volar piece by a cut which extends as far as the cavity of the mid-carpal joint. By pulling the dorsal and volar pieces apart a clear view of the carpal screw is obtained.

The action of the carpal screw must be studied on a different preparation. The hand and carpus are carefully disarticulated from the forearm at the radio-carpal joint, and the lunate and triquetrum are separated in one piece from the rest of the carpus. It is then easy to demonstrate that dorsiflexion of the hand upon the lunate-triquetrum—the parts being gently pressed together—

Text-fig. 4. The proximal articular surfaces of the hamate, capitate and navicular bones, showing the screw surface described in the text. The preparation has been arranged with the hamate bone above for reasons of perspective. Left hand, dorsal aspect. ×2.

will bring about an approach of the navicular to the lunate, while volar flexion has the contrary effect. In other words, the parts separated by our dissection are screwed together in dorsiflexion and loosened in volar flexion: and this is the mode of action of the carpal screw.

It is clear that the bones of the carpus are welded together in dorsiflexion; that a closer packing replaces a looser packing; and that this closer packing is brought about by a two-stage process. The first stage is that in which the navicular is brought to rest on the distal row, and so made effectively one with it; the second stage is that in which the lunate, and with it the triquetrum,

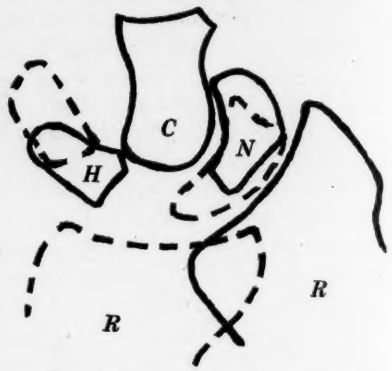
is screwed against and brought to rest upon the navicular, which thus fulfils its role as a link between the two rows. By stating some of these facts rather differently—by saying that the second stage is one in which the triquetrum is screwed towards the navicular, pushing the lunate with it—the resemblance of the whole mechanism to that of a screw-vice, or screw-clamp, is made more obvious. The first stage of dorsiflexion is, then, one in which the clamp is set up, or constituted, by fixing the navicular, the fixed jaw of the vice, to the distal row, which acts mechanically as the base of the vice. In the second stage, the hamate acts as a screw to pin the lunate against the fixed jaw, and to hold it there for so long as dorsiflexion is maintained. Since the triquetrum, lunate and navicular are normally joined by quite short interosseous ligaments, this concept of the carpal mechanism may seem to be somewhat abstract: that it has some basis in practice will be shown by applying it to the problems of the reduction and the maintenance in position of a dislocated lunate bone. In the meantime it is necessary to consider the evidence furnished by the living subject for the closer packing of the bones of the proximal row in the position of dorsiflexion. This evidence is furnished by considering the proximal row in radial and in ulnar deviation of the hand.

THE PROXIMAL ROW IN RADIAL AND ULNAR DEVIATION

Superimposed tracings of the proximal row in radial and in ulnar deviation are shown in Text-fig. 5. As before, the capitate bone is taken to be fixed, as if the hand were kept at rest and the forearm were the visibly moving part. The outline of the lower part of the radius is also shown. This method of superimposition shows that there is a gradient of angular velocity in radio-ulnar motions also. The hand being taken as fixed, the proximal row rotates round the head of the capitate and so also do the radius and ulna; but they rotate to a greater extent than does the proximal row. The muscular forces act primarily (in the case considered) upon the forearm bones; they communicate their motion in lesser degree to the proximal row: but, it must be specially noted, the three bones of the proximal row receive this communicated motion in varying amounts, a fact upon which the argument of the present section depends. In the passage from full radial deviation to full ulnar deviation, the triquetrum moves most. The navicular moves least, a fact that can be ascribed to its manner of articulating with the other wrist bones, and to the restraint put upon it by the interosseous ligament connecting it with the capitate and by the fibres of the radial collateral ligament—the last, it is to be remembered, being under tension in the movement considered. The movement of the lunate around the head of the capitate is intermediate in amount between those of its neighbouring bones. The result of these differences of movement is that the proximal bones become more loosely packed: the interosseous ligaments connecting them are arranged to allow this. The behaviour of the bones of the proximal row in ulnar deviation (from the extreme radial

position) may be summed up by saying that they "flow" round the head of the capitate. Their loosening from one another is a necessary concomitant of this flow. In radial deviation they become, of course, more closely packed again.

If, then, the carpal bones are brought more closely together and kept so by the act of dorsiflexion, it follows that the act of rocking the hand from side to side upon the forearm should be more difficult the more the hand is dorsiflexed; and possibly the same should be true in volar flexion as well, for the carpal bones being loosened in this posture the amount of further loosening to admit of the flow of the proximal bones around the capitate will be so much less. It is easy to verify upon the living subject that both these conclusions are true: it is in fact impossible to rock the hand from side to side in full dorsiflexion, and any attempt to do so only results in movements at the radio-ulnar joints: this is true for both active and passive rocking. Moreover, it is well known that any attempt to dorsiflex the fully "deviated" hand has the result of bringing the hand into a more central alignment with the forearm. On the other hand, dorsiflexion is no bar to passive twisting movements at the radio-carpal joint—that is, to movements of the whole carpus about an axis passing longitudinally along the forearm. This is quite consistent with the theoretical mechanism which has been described above.



Text-fig. 5. Relative displacements of the radius and proximal carpal bones on the capitate in the passage from full radial deviation (full line) to full ulnar deviation (broken line). The lunate bone has not been drawn in this series of superimposed tracings from radiographs. The angular velocity of the radius is about twice that of the triquetral and about three times that of the navicular. Left hand, dorsal view.

THE REDUCTION OF A DISLOCATED LUNATE BONE

It is a direct inference from the account which I have given of the working of the carpus, that the lunate bone is free from the pressure of its neighbours in volar flexion but is caught and held by them as the hand is brought into dorsiflexion. Accordingly, it should be possible to restore the normal alignment of the bones of the proximal row by flexing the wrist and then dorsiflexing it: for this manœuvre would bring the jaws of the clamp beside the lunate, and it would be caught and pressed between them as the hand was dorsiflexed, and so brought back into line with them. It is a further inference that the best position in which to keep the carpus so reduced in plaster would be the dorsiflexed position; for here the whole carpus acts as a splint to the lunate, even if the navicular or triquetrum be broken.

I am indebted to Mr F. W. Holdsworth, F.R.C.S., for putting this matter to the test at the Fracture Clinic of the Sheffield Royal Infirmary. He has indeed already read a summary of his results before a recent meeting of the British Orthopaedic Association (Holdsworth, 1940).

Eighteen cases of anterior dislocation of the lunate (perilunar dislocation) have been treated at the above clinic in the past three years. Eleven of these have occurred within the past two years. Two of these eleven had not come for treatment until over 14 days after injury, and manipulative treatment was deemed to be out of the question for them. The remaining nine cases were treated by the method which has just been suggested: immediate reduction was achieved in all these, that is, in every case that presented itself less than 11 days after injury within the past two years. All those treated were workmen; and they have been able to resume their work with ease and satisfaction to themselves. An analysis of the nine cases treated by the flexion-dorsiflexion method is given in Table 1. The table shows that all possible combinations of fracture of the navicular and triquetrum have been dealt with: the numbers treated are as yet too small to allow any conclusions to be drawn from the relative frequencies of the four types listed, other than that those frequencies are consistent with the statement that fracture of the triquetrum is quite independent of fracture of the navicular, and conversely. The plate shows an example of the reduction of a simple dislocation of the lunate bone.

Table 1. *An analysis of the fracture of bones of the proximal carpal row as they were found in nine cases of dislocation of the lunate bone reduced by the method described in the text*

	Navicular fractured	Navicular not fractured
Triquetrum fractured	1	3
Triquetrum not fractured	2	3

Technique of manipulative reduction

The patient is fully anaesthetized. The surgeon grasps the forearm of the injured side with one hand. He uses the thumb of this hand to exert a *gentle* steadying pressure upon the dislocated bone. The pressure must be gentle to avoid injury to the overlying median nerve. The surgeon's other hand grasps the injured hand, and the manipulation proceeds as follows. Volar flexion is carried out slowly. The last stage of volar flexion is completed suddenly and sharply and with force, and then the hand is immediately, quickly and firmly dorsiflexed. The reduction has now been effected: this is, of course, to be confirmed by radiography. The manipulation takes about a minute to accomplish. Slight traction should be made on the hand while it is being carried out; for the object of the volar flexion is to draw the carpus, and in particular the head of the capitate, over the lunate bone.

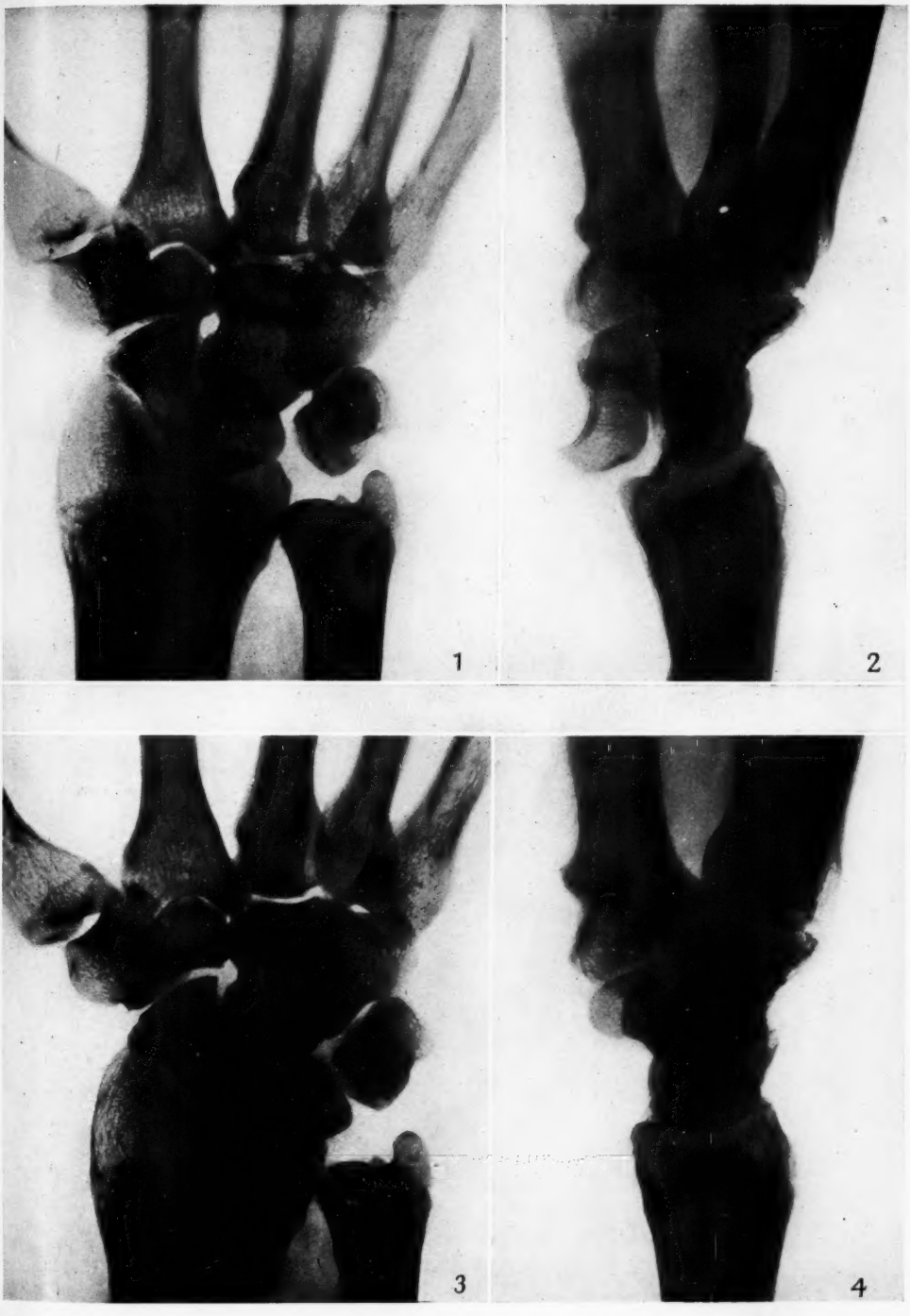
After reduction, the limb should be put up in plaster, the hand being in a position just short of the fullest dorsiflexion.

Discussion of the technique

There are three types of simple manipulative reduction of a perilunar dislocation: that is, reduction without such extraneous aids as, for example, the needle used by Müller (1938) to push the lunate back. The three types are: simple traction (Porritt, 1938; Stevenson, 1940); dorsiflexion followed by volar flexion (Jeanbrau, 1921; Watson-Jones, 1929; Douglas, 1936); and volar flexion followed by dorsiflexion, suggested in this paper. All three methods have been successful in some cases, for confirmatory radiography has been used. The special interest of the method I have devised lies in the support it gives to my account of the mechanism of the wrist-joint. Its worth as a routine technique must be judged by the experience of surgeons: it is quick, and can be tried before other methods are resorted to. No manipulation will succeed if the space for the lunate bone has become filled with fibrous tissue: this was present in the two late cases spoken of above, which had to be treated operatively.

THE ROLE OF THE LUNATE BONE

Monnard (1925) has made a study of the effect of removing the lunate bone from the dynamic point of view. His results have a bearing upon the significance of the carpal mechanism. He had fourteen cases to deal with: in eight the lunate had been taken away, in six it had been left. In all these cases he compared the range of angular displacement and the dynamometric efficiency of the affected side with those of the sound side. In those from whom the lunate had been removed there was a retention of 57 % of angular movement (flexion-dorsiflexion) and 68 % of power of grip. In those in whom the lunate had been left in the wrist there was 90 % of angular movement retained together with 85 % of power of grip. These are, of course, average figures. They are objective evidence of the truth of the statement made by many patients from whom the lunate has been removed: that they can use their wrists, but suffer from a "lack of power" in them. So far as they go, these observations are consistent with the essential dynamic thesis of this paper: namely, that the side-to-side pressures in the carpus (of the bones against each other) are just as important functionally as the longitudinal pressures (of the carpus against the forearm). The substitution of fibrous tissue for the lunate bone destroys the effect of the lateral pressures engendered by the carpal clamp. The lunate may be regarded as a separated portion of a single skeletal complex represented by the proximal carpal bones. Such a complex is found as a single bone in bats (Chiroptera); approaches to it are found amongst carnivores (fusion of scaphoid with lunate), and very occasionally in man (fusion of triquetrum with lunate). The parts of the complex are virtually united in man in full dorsiflexion, and are broken apart in all other positions. Dislocation of the lunate represents a



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fracture of the complex; and it is not surprising that the fracture should sometimes spread so that it involves the marginal parts of the complex as well: that is, produce a fracture of the navicular, or of the triquetrum, or even both.

SUMMARY

1. The carpus without the lunate bone is a screw-vice or screw-clamp. This clamp comes into action in dorsiflexion and then grips the lunate firmly.

2. This conception of the mechanism of the carpus is applied to the manipulative reduction of a dislocated lunate (perilunar dislocation) and to the retention of the limb in plaster. The manœuvre is essentially one of volar flexion followed by dorsiflexion. Nine successful cases are reported.

3. The displacements of the bones of the proximal carpal row are due essentially to their position in a proximo-distal gradient of angular velocity. The gradient arises from the viscous nature of the synovial fluid of the wrist-joints.

4. The basic movements referred to in (3) are modified in accordance with the part each bone plays in the clamp-mechanism.

5. The mechanics of the wrist given here are the foundation of the movements of the individual bones previously described by Fick and other investigators.

I wish to thank Prof. F. Davies for his encouragement and helpful questioning. I have already mentioned my debt to Mr F. W. Holdsworth, of the Sheffield Royal Infirmary. My special thanks are due to Dr J. L. A. Grout, Lecturer in Radiological Anatomy, and to Mr A. L. Watson, Radiographer to the Sheffield Royal Hospital, for their willing help in the making of the stereoscopic and other pictures used in this investigation.

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EXPLANATION OF PLATE 1

Reduction of a simple dislocation of the lunate bone by the method described in the text. Right hand, dorsal and lateral views. (1) and (2), before reduction; (3) and (4), after reduction.

THE ANGIOARCHITECTURE OF THE THALAMUS IN THE RABBIT

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INTRODUCTION

Aitken, in a paper published in 1909, described the larger blood vessels of the lenticular body, while Finley (1936) described the arteries, veins and capillaries of the substantia nigra. Apart from these investigations no attempt seems to have been made to describe the circulatory system of a specified subcortical area of the cerebrum as a whole. A great many publications are concerned with the arterial supply alone and a small number with the venous drainage; more comprehensive studies have apparently been neglected. Pfeifer (1930), whose work has thrown so much light upon the anatomy of the blood vessels of the brain, has confined his investigations mainly to the vessels of the cortex and the white matter, and he deals with the whole subject largely from the viewpoint of tissue morphology and general angioarchitecture.

The bearing of the present investigation on a number of important questions may be briefly noted as follows:

(1) If there actually exists a principle, first formulated by Duret (1874), which governs the relationship between arteries and neural mechanisms and establishes a constant neurovascular pattern in the phylogenetic series, this pattern must obviously be of material aid in identifying nerve centres which are otherwise difficult to homologize. This method, for instance, has been attempted by Abbie (1934) who claims that the vascular supply of the various parts of the basal ganglia is constant from fish to man, and on this assumption draws far-reaching conclusions on the relationship between the hypopallium of reptiles and the basal ganglia of higher vertebrates.

(2) As the subcortical nerve centres represent fairly distinct anatomical units, quantitative and qualitative differences in their vascular supply and angioarchitecture, like those encountered among the various cortical areas, are to be expected, and they probably have a similar significance for comparative studies.

(3) Some pathological conditions, probably of a chronic infective or degenerative nature, can hardly be fully investigated (as, for instance, a number of perivascular lesions described by Finley (1936)), because the nature of the vessels involved has not been determined. According to Finley this is mainly due to the lack of knowledge of the angioarchitecture of the affected region. Difficulties of this kind can probably be overcome, even if only to a limited extent, if typical differences in the arrangement and the distribution of arteries and veins can be demonstrated systematically in various regions.

Naturally the present study has a direct bearing only upon questions of

comparative anatomy. It is to be expected, however, that some features encountered in the phylogenetically older parts of the brain will be met with in lower and higher mammals alike, and that the knowledge gained on lower forms and simpler structures will facilitate similar investigations in the more elaborate brains of the higher primates, including man.

MATERIAL AND METHODS

Eight rabbit brains were used for this investigation. In four brains the course of the vessels was followed under the binocular microscope from the circle of Willis up to the basal and lateral surface of the thalamus, two of the animals having been previously injected with 15 c.c. of a 2% solution of trypan blue. India-ink injections are less suitable for the study of surface vessels in a smaller brain, as the vascular pattern is so dense that the course of the main vessels becomes obscured and it is practically impossible to follow their various ramifications with accuracy. In the uninjected brains the arteries appear as white, glistening vessels under the microscope, the veins appearing dark by contrast. The four remaining brains were cut into serial sections at a thickness of 250 μ after the animals had been injected through the aorta with carmine gelatine, india-ink gelatine, india ink diluted with water, or trypan blue. By far the best results were obtained with the aqueous india-ink injection, which penetrated into the whole capillary network, except for a few small patches. In these areas, however, the walls of the blood vessels could be seen quite easily with the use of a high-power objective and a properly adjusted light, and indeed the uninjected patches were of some help in determining the boundaries of the main vascular territories immediately adjacent to them. The india-ink series was counterstained with van Gieson. The carmine-gelatine and india-ink gelatine masses penetrated into the larger vessels only, although the vascular system had been washed out with hot saline solution. The trypan-blue series finally showed the vascular system without distortions (which can hardly ever be avoided when other injection masses are used) and brought the venous pattern out more clearly than the arterial pattern. The present investigation is largely based on the aqueous india-ink series, and a projection reconstruction comprising twenty-five sections was made by tracing practically every larger visible vessel from the interior of the thalamus to its periphery.

To compare various features encountered in the brains of the rabbits, two monkeys' brains were used, one of which had been injected with carmine gelatine and the other stained with Pickworth's stain (1934). For further comparison the brain of a rat was stained with picric acid, light green and acid fuchsin, a combination which demonstrates the capillary pattern beautifully.

EXTRACEREBRAL THALAMIC ARTERIES

Two main groups of vessels can easily be identified, (1) a dorsolateral, and (2) a ventromedial group (Fig. 1).

(1) *Dorsolateral group*

On the dorsal and lateral surfaces of the thalamus three groups of arteries are visible after the removal of the hemispheres, which may be called the anterior, middle and posterior superior thalamic arteries. They arise at very acute angles from the posterior cerebral artery, an artery which is either the continuation of the vertebral artery, or, if the connexion between the posterior cerebral and the vertebral artery is reduced in size, of the posterior communicating artery. As these vessels lie on the dorsolateral surface of the thalamus

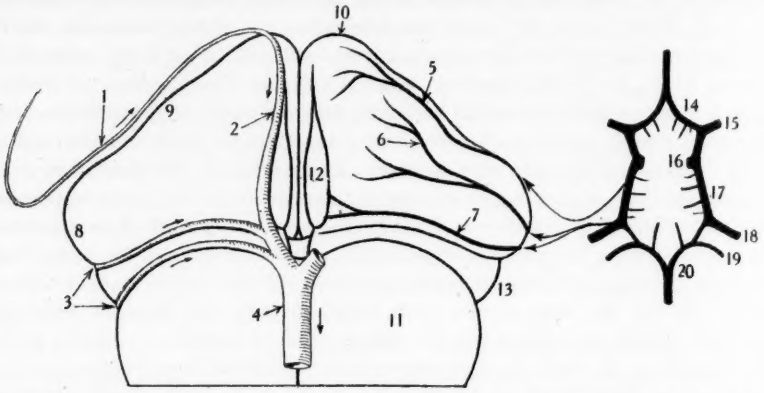

Fig. 1. Diagram showing the origin from the circle of Willis of the arteries on the dorsal and lateral surfaces of the thalamus. 1, chorioidal and terminal vein; 2, small vein of Galen; 3, veins in pretectal area; 4, vein of Galen; 5, 6, 7, dorsolateral thalamic arteries, anterior, middle and posterior group; 8, lateral geniculate body; 9, lateral tubercle; 10, anterior tubercle; 11, superior colliculus; 12, habenular nucleus; 13, medial geniculate body; 14, anterior cerebral artery giving rise to the anterior inferior thalamic arteries; 15, middle cerebral artery; 16, internal carotid artery; 17, posterior communicating artery giving rise to the middle inferior thalamic arteries; 18, posterior cerebral artery; 19, anterior cerebellar artery; 20, vertebral artery giving rise to the posterior inferior thalamic artery.

and run towards the midline, their course becomes tortuous, thus spreading the origins of the parenchymal end-arteries, to be described later, over a wide area. It may be noted that the anterior collicular arteries arise with the posterior cerebral artery by a short common trunk, the next vessels to be given off being the thalamic arteries. The posterior collicular artery is a branch of the superior cerebellar artery.

(*a*) *The anterior superior thalamic artery* is the smallest thalamic vessel. It may be an independent branch of the posterior communicating or a branch of the middle superior thalamic artery. In some cases it arises from the anterior chorioidal artery which in the rabbit seems to be a rather constant branch of the posterior communicating artery (and not of the anterior cerebral or internal carotid). The anterior superior thalamic artery runs towards the region of the anterior nucleus of the thalamus.

(*b*) ***The middle superior thalamic artery*** is larger than the anterior vessel and lies largely on the lateral tubercle of the thalamus. This artery, too, curves round the lateral geniculate body before reaching the dorsal surface of the thalamus.

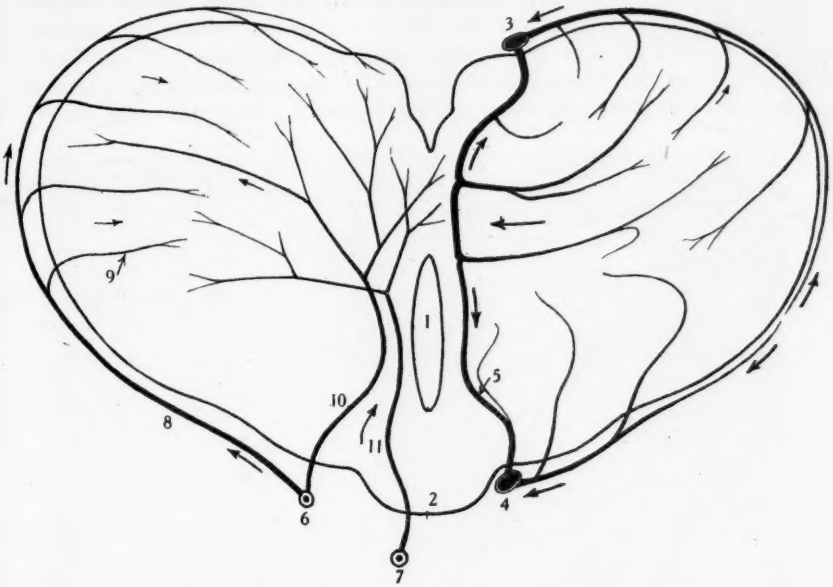

Fig. 2. Diagram showing the different arrangement of arteries and veins in the thalamus of the rabbit. 1, third ventricle; 2, mamillary body; 3, small vein of Galen; 4, basal vein; 5, anastomotic chain, connecting 3 and 4; 6, posterior communicating artery; 7, vertebral artery; 8, dorsolateral artery; 9, parenchymal arteriole, arising from 8; 10, middle inferior thalamic artery; 11, posterior inferior thalamic artery.

(*c*) ***The posterior superior thalamic artery*** follows an almost transverse course, forming a fan-shaped pattern with the other two vessels. It lies behind the lateral geniculate body and gives branches to the posterior portion of the habenular nucleus.

The extracerebral course of the dorsolateral vessels is rather long, especially that of the anterior superior thalamic artery in cases in which it forms the continuation of the anterior chorioidal artery.

(2) *Ventromedial group*

The extracerebral course of this group of vessels is short, since they enter the brain substance almost immediately after arising from the circle of Willis. They, too, can be subdivided into three arteries or rather into three groups of arteries:

(*a*) *Anterior inferior group*. This consists of numerous arteries of very small calibre which arise from the anterior cerebral artery in the region of the optic chiasma and enter the hypothalamus.

(*b*) *Middle inferior group*. This arises from the posterior communicating artery and enters the brain substance between the tuber cinereum and the cerebral peduncle (Figs. 2, 3).

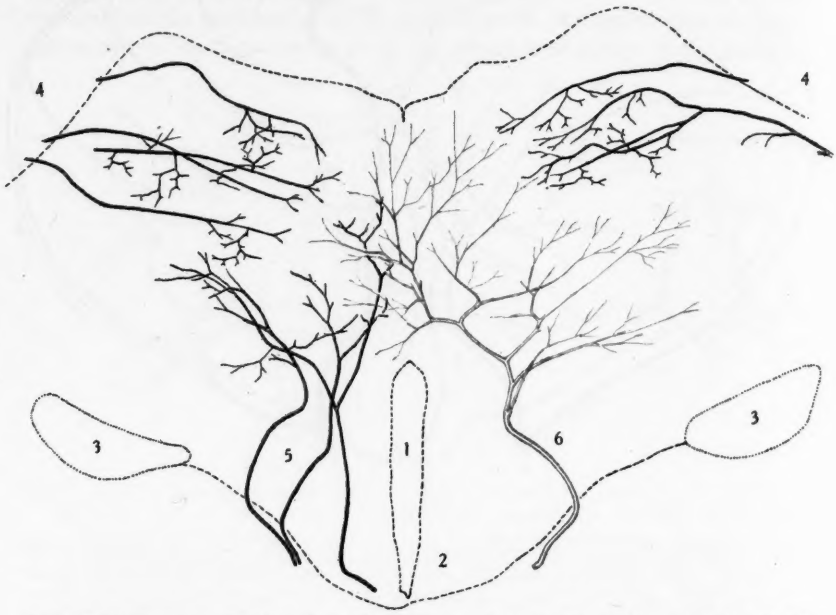

Fig. 3. Projection reconstruction, showing arteries in rostral half of the thalamus (sections 21–31) and the general asymmetry of the arterial supply. 1, third ventricle; 2, tuber cinereum; 3, optic tract; 4, dorsolateral parenchymal arteries; 5, middle inferior thalamic arteries on the left; and 6, single artery on the right side, trespassing across the midline of the thalamus. (×10.)

(*c*) *Posterior inferior group*. These vessels take origin from the posterior cerebral or the vertebral artery.

The arteries of the second and third groups are asymmetrically developed, the larger vessels taking over the supply of the midline nuclei and their branches sometimes trespassing across the midline, as will be shown later.

ANGIOARCHITECTURE OF THE THALAMUS

Before the intrathalamic vessels are described a short account may be given of the nuclear configuration of the thalamus, which is largely based on the illustrations in the atlas by Winkler & Potter (1911) and Rose's monograph (1935).

The thalamus on each side in the rabbit is a pyriform body, the smaller, anterior pole pointing forwards and medially, and the larger, posterior pole (which is continuous with the lateral geniculate body) backwards and laterally. In coronal sections through the thalamus two main areas can be identified, a roughly circular region situated medially and subdivided by medullary laminae, and a lateral, sickle-shaped field which holds the rounded area in its concavity. The lateral area comprises rostrally the reticular nucleus (which separates the remaining nuclei from the internal capsule and, rather incompletely, from the hypothalamus), and also, more caudally, the lateral and medial

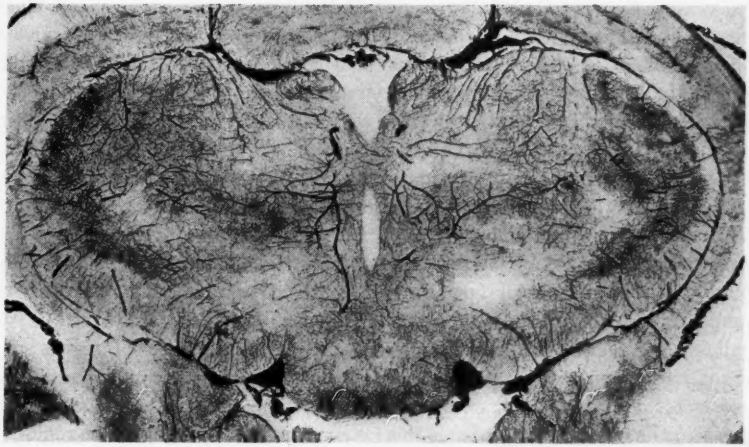

Fig. 4. Section through the caudal half of the thalamus of a rabbit (no. 34) injected with aqueous india-ink in solution ($250\mu \times 9$) (compare with Fig. 2). The two symmetrically placed dark areas on either side represent the lateral geniculate bodies. In the right geniculate body, branches from a parenchymal arteriole are given off at right angles in opposite directions, resembling the cortical pattern of arterial distribution. The large arterial branch in the middle of the right half of the picture supplies the ventral nucleus, whereas, on the left side, this nucleus is supplied by vessels of the dorsolateral group. The paraventricular anastomotic chain of veins is partly visible on the left side of the midline. The basal veins and the small veins of Galen are visible as dark patches.

geniculate bodies (Fig. 4). Three of the nuclei, the anterior, lateral and habenular, project on the dorsal surface of the thalamus. The anterior, medial and ventral nuclei are connected through the connexus interthalamicus with those of the opposite side. The ventral nucleus forms the larger part of the base of the main inner nuclear mass. A group of cells occupying the topographical position of the centre median nucleus lies between the medial, ventral and lateral nuclei, while the posterior nucleus is situated above the posterior portion of the ventral nucleus, extending far backwards under the anterior margin of the superior colliculus. On the dorsal surface of the thalamus the lateral geniculate body and the lateral nucleus are separated from the superior

colliculus by the pretectal area. The cavity of the third ventricle is partly obliterated by the large connexus interthalamicus. In this complicated nuclear mass the intrathalamic vessels are arranged in the following manner.

(1) *The intrathalamic course of the dorsolateral thalamic arteries* (Figs. 2, 3)

After forming a rather uniform pattern by dividing and subdividing on the surface of the thalamus at acute angles, the dorsolateral thalamic arteries break up into their intrathalamic branches. There is a distinct tendency for these vessels to enter the thalamus perpendicularly to the surface, but there seems to be in many instances some sort of compromise between the surface type of ramification, where branches arise at acute angles, and the rectangular origin of the branches which enter the thalamus. On the whole, however, the perpendicular mode of entry governs the course of the vessels within the substance of the thalamus, as the vessels which enter the thalamus at lower levels have a horizontal course or may even be directed inwards and upwards, whereas the arteries arising more on the dorsolateral and dorsal surface assume a downward direction which becomes gradually more distinct. Not only has the direction of the primary intrathalamic vessels a certain relationship to the configuration of the dorsolateral surface of the thalamus, but many of the secondary rami, too, show some interdependence in so far as they tend to run parallel to the surface of the thalamus. There are also a number of vessels with a slightly recurrent course. In general, the intrathalamic arrangement of the dorsolateral arteries bears some faint resemblance to the arrangement of arteries in the cerebral cortex, and this becomes more apparent in the region of the colliculi; it is quite different from the distribution of the thalamic arteries which arise from the ventromedial group. The branches given off in the deeper regions of the thalamus have a different manner of ramification which will be described later in more detail. The vessels just described can hardly be subdivided further, as their origins are widely scattered over the whole surface of the thalamus and the lateral geniculate body.

(2) *The intrathalamic course of the ventromedial thalamic arteries*

These vessels comprise three distinct groups, according to their origin at the base of the brain (as already described), their size, and their distribution. They arise from rather distant points of origin, involving practically the whole circle of Willis, and they all converge towards the centre of the thalamus.

(*a*) *Anterior inferior group.* This group can only doubtfully be regarded as "thalamic" in the proper sense. It comprises numerous small vessels, in fact, the smallest of the arteries to be described, which run upwards and slightly backwards. It is questionable, however, whether these arteries actually reach the basal layers of the anterior nucleus or whether they exhaust themselves in the dorsal levels of the hypothalamus.

(*b*) *Middle inferior group*. This group is distinctly asymmetrical. There was, in one specimen, one very large vessel on the right side and several small vessels on the left side. The amount of blood carried by the arteries on the left side was however much smaller.

(*c*) *Posterior inferior group*. These arteries, too, are asymmetrical. This asymmetry will be described later in connexion with the blood supply to the ventral nucleus. Whereas the branches of the second group extend mainly upwards and forwards, the most caudal branches of the posterior group extend backwards and downwards on either side of the aqueduct.

(3) *The territories of supply of the thalamic arteries*

The territory of the ventromedial group is wedge-shaped, the thin end of the wedge being directed dorsally in the midline of the thalamus and the base corresponding to the base of the brain. The territories of the dorsolateral group occupy the region between the oblique lateral surfaces of the wedge and the dorsolateral surface of the thalamus. The base of the wedge is narrower in front than behind. There are numerous irregularities modifying the contour of the boundary between the two territories, as protrusions of the various areas penetrate into the neighbourhood, interlocking with corresponding extensions of the adjacent territories. It is convenient to distinguish processes of the first order, second order, and so forth. An extension of the dorsolateral group towards the midline, for instance, which fills the gap between the territories of the middle inferior and posterior inferior thalamic arteries, must be considered as a protrusion of the first order, as it is of considerable size and separates areas of large vessels. Another protrusion of the first order, directed into the opposite direction, is an area belonging to the middle inferior thalamic artery, which forms a long finger-like extension towards the dorsolateral surface, nearly interrupting the dorsolateral areas in the regions of the more superficial portions of the reticular nucleus. Extensions of the second order which interdigitate with one another are represented by the smaller twigs of the arteries and make the boundaries between the main vascular areas still more irregular.

It was possible to determine in the anterior region of the thalamus the boundaries of the two main regions quite accurately, as the injection had penetrated practically everywhere into the capillaries of the dorsolateral arteries but only into that part of the central area supplied by the middle inferior thalamic arteries. In the completely injected dorsolateral territories every vessel, including the precapillaries and capillaries, was well outlined, the ramifications being directed towards the centre of the thalamus, whereas in the incompletely injected ventromedial area the branches of the arteries—which could be clearly seen when the light was properly adjusted—were directed towards the periphery.

(4) *Arterial supply of the thalamic nuclei*

As was suggested by the description of certain vascular asymmetries, the relationship of the thalamic nuclei to the ventromedial and dorsolateral areas must be expected to differ on the two sides. The somewhat diagrammatic description of the configuration of the vascular areas which has been given requires to be qualified by reference to the following disturbing factors:

(1) Partial or total asymmetry. The minor asymmetries are a very common feature and will not be specifically mentioned.

(2) Vascularization of parts of the deep-lying nuclei by vessels of dorsolateral origin.

(3) Vascularization of parts of the superficial nuclei by arteries of ventromedial origin.

The several nuclei of the thalamus were found to be vascularized in the following manner:

(*a*) *Nucleus anterior.* Superficial part, comprising both the nucleus anterodorsalis and anteroventralis, by the anterior superior thalamic artery. Deep layers of the nucleus—anterior rami of the inferior middle thalamic artery.

(*b*) *Medial nucleus* (*n. dorsomedialis*). Medial branches of the middle inferior thalamic artery, which are given off before the vessel enters the anterior nucleus from below. On the right side some branches of the dorsolateral group of arteries were seen entering the lateral layers of the dorsomedial nucleus.

(*c*) *Cell groups occupying the topographical position of the centre median nucleus.* Very long dorsolateral arteries reach the lateral extremity of this element from the surface of the thalamus. The remaining part is supplied by the middle inferior thalamic artery.

(*d*) *Nucleus ventralis lateralis* ("N. ventralis *a*" of Winkler and Potter). Left side: middle superior and posterior superior thalamic arteries. Right side: large branch of the posterior inferior thalamic artery. Remaining parts of the nucleus ventralis: posterior superior thalamic artery.

(*e*) *Lateral nucleus.* Largely supplied by the middle superior thalamic artery, some branches being derived from the ventromedial group. The former represents the large territory previously referred to which fills the gap between the area of the middle inferior and posterior inferior thalamic arteries. Posterior portions of lateral nucleus: posterior superior group of vessels which penetrate and supply the lateral geniculate body before breaking up into deeper branches.

(*f*) *Posterior nucleus.* Posterior branches of the posterior inferior thalamic arteries. This nucleus has apparently the most symmetrical supply of all the thalamic nuclei, lying far away from the "disturbing centre" of the connexus interthalamicus and the adjacent anterior and dorsomedial nuclei.

(*g*) *Habenular nucleus.* Anterior portion: middle and anterior superior thalamic arteries. Posterior portion: posterior superior thalamic arteries.

(*h*) *Reticular nucleus.* Formatio reticularis *a*: middle inferior and posterior

inferior thalamic arteries. Formatio reticularis *b*: branches of the dorsolateral group of vessels. Some parts of the upper division (reticularis *b*), however, receive their arterial supply by long branches of the middle inferior thalamic artery which have already been referred to and which intrude deeply into the territory of the dorsolateral group.

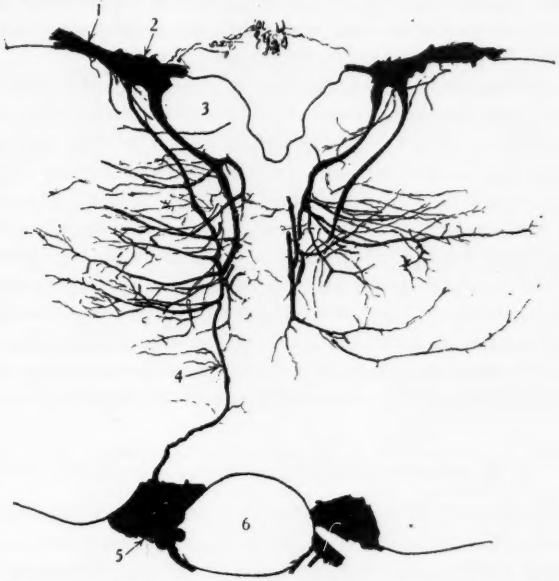

Fig. 5. Projection reconstruction, showing arrangement of veins in the caudal part of the thalamus (sections 30–40). 1, pretectal vein; 2, small vein of Galen; 3, habenular nucleus; 4, anastomotic chain, connecting 2 and 5; 5, basal vein; 6, mamillary body. (×10.)

(5) *Arrangement and territories of the intrathalamic veins. Topographical differences between arteries and veins*

The arrangement of the thalamic veins is entirely different from that of the arteries in the upper and medial portions of the thalamus but shows some resemblances in the remaining parts (Fig. 5). For the purpose of our description, each coronal section through the thalamus may be subdivided into four quadrants, two upper and two lower. These quadrants overlap very considerably and their boundaries are somewhat arbitrary, though it would seem that much more blood is carried through the upper medial quadrant than the remaining quadrants. Its veins convey blood from the larger part of the upper half of the thalamus and at first run downwards and inwards, but they curve gradually upwards to form a row of veins running parallel to the wall of the third ventricle and the median plane. The terminal portions of these large veins (especially in the posterior part of the thalamus) travel in the medullary

laminae separating the habenular nucleus from the medial and lateral nuclei. They open finally into the small vein of Galen. The large paramedian veins are present throughout the thalamus in an anteroposterior direction but are more densely arranged in its anterior and posterior regions, so that it is possible to identify an anterior and a posterior group. The former is directed slightly forwards, to join the beginning of the small vein of Galen, while the posterior group is directed backwards and opens into the small vein shortly before it joins its fellow to form the large vein of Galen.

The two lower quadrants are drained by small veins the course of which is S-shaped and resembles to a certain extent the distribution of the ventromedial group of arteries. They open into the basilar vein. There are large anastomoses between the veins of the two inner quadrants, which form a vertical anastomotic chain on either side of the third ventricle between the small vein of Galen and the basilar vein. Larger veins leave the thalamus through the posterior perforated space. They run in association with the posterior inferior thalamic arteries and are connected with one another by short anastomoses lying immediately underneath the posterior perforated space and outside the brain.

The superior lateral quadrant is drained by veins which follow closely the course of the dorsolateral group of thalamic arteries; this feature adds further to the similarity between the vascularization of this territory and that of the cortex.

(6) *Morphological differences between arteries and veins*

The topographical differences between arteries and veins which have been brought out by the foregoing description are much more obtrusive than the morphological differences, the latter being apparently dependent to a certain extent on the situation of the vessels within the thalamus. A knowledge of the distribution of arteries and veins may therefore be of great help in determining to which category a vessel belongs. A reliable diagram can only be made, however, if the morphological as well as the topographical differences are taken into account. In some instances the diagnosis is so difficult that every vessel must be traced to a surface vessel before the question can be definitely settled. This, indeed, was done with practically every vessel before Table 1 could be compiled.

It is not always possible, in spite of all the differences enumerated in Table 1, to determine the nature of a vessel, for there are many kinds of transitional forms. Morphological differences between the vessels of the various regions are referred to in the table: these differences exist within the thalamus and to a still more marked degree between the thalamic vessels and those of other parts of the brain. Arteries with a zigzag course, for instance, which must not be mistaken for the zigzag course of veins, were observed in the cerebral cortex of the rabbit (as previously in the cortex of the cat and in man by Pfeifer), but they were never seen in the rabbit's thalamus. We will try to explain the regional differences of the arteries in the discussion of our findings.

Table 1. ***Comparison of intrathalamic arteries and veins***

	Arteries	Veins
General appearance of main stem	May take a perfectly straight course over a considerable distance (from 1 to 1½ mm.)	Straight course observed only in very large, easily recognizable veins of a size not attained by arteries; otherwise by the veins of the upper lateral quadrant only
Tortuosity	Can be traced, if present, to a sudden narrowing of the calibre in one or several places and a surrounding ring of contraction of the muscular wall	Cannot be traced, if present, to a contracted part of the wall but to a congested vein into which the tortuous vessel opens
Wall	Thick in relation to the calibre of the vessel. Stains red or yellowish with van Gieson in larger arteries. Shows a longitudinal and circular structure particularly recognizable where the vessel has been cut	Practically invisible
Perivascular space	Very narrow and not at all visible in smaller arteries. Where the artery is tortuous, the tortuosity does not affect the perivascular space. Perivascular spaces more frequent in ventromedial group of arteries	Very large, especially around larger veins near their termination in parent vessels
Number of rami	Large portions of main stem without any rami frequently observed	Branches given off at frequent intervals, except near the point where large tributaries join a parent vessel
General type of ramification	(*a*) Tendency to ramify in all directions and to cover a maximum of space in all three dimensions (*b*) The smaller rami repeat the pattern of ramification of the main stem and of larger branches, the calibre of the branches of first, second, etc. orders, diminishing gradually. Diminution of calibre reached gradually in successive steps ("Allmählicher Kaliberabfall", Pfeifer)	(*a*) Tendency to ramify chiefly in one plane (*b*) Ramification may be confined to branches of first order, where large stems give rise to twigs which are very small in proportion to the main stem and may be given off at very frequent intervals (feather-like pattern). Diminution of calibre reached abruptly in one step ("Steiler Kaliberabfall", Pfeifer)
Cone of origin of branches	Base of cone small	Base of cone of origin very large. Cones superimposed on main stem at frequent intervals give the impression of a zigzag course of the main stem or its larger branches
Direction of branches	Given off frequently at right angles or angles of nearly 90°. Recurrent branches sometimes occur in the lateral superficial layers of the thalamus	Given off, as a rule, at acute angles, though small "disproportionate" twigs may join a large stem at right angles

(7) *The capillary network of the thalamus*

This may be dismissed briefly, as the accompanying microphotograph (Fig. 4) is self-explanatory. The nuclei may be arranged according to the richness of the capillaries in the following order: (1) nucleus anterior, (2) lateral geniculate body, (3) nucleus ventralis, (4) nucleus habenulae, (5) other nuclei.

The density of the capillary network in the anterior nucleus in the monkey

(Fig. 7), which was mentioned in a previous communication (Schlesinger, 1939), seems to be a fairly constant feature, since it can also be observed in the rat.

A few words may be said about the supraoptic and paraventricular nuclei in the rabbit and rat, as their angioarchitecture has been recently described in the monkey by Finley (1939). The capillary loops are very narrow in the rabbit and very densely arranged, the capillary calibre being small. Many loops run parallel to the surface of the optic tract, as seen in transverse section, although

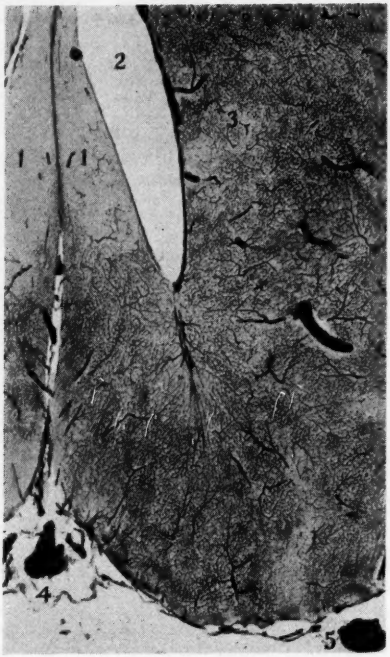

Fig. 6. Coronal section through the brain of a rhesus monkey at the level of the anterior horn of th lateral ventricle, showing blood vessels radiating towards the ventricle from the periphery (carmin-gelatin injection, 250μ × 6). 1, septum pellucidum; 2, anterior horn of lateral ventricle; 3, caudate nucleus; 4, anterior cerebral artery; 5, middle cerebral artery.

this pattern is less obtrusive than in the monkey. The paraventricular nucleus has a very dense capillary network in the rat. In our injected specimens it was not so well outlined.

EXTRACEREBRAL THALAMIC VEINS

Some of these veins form a network which is situated in the subarachnoid space overlying the thalamus and which is connected with the veins of the chorioid plexus of the third and lateral ventricles and the terminal vein; other

extrathalamic veins are represented by three large venous channels which run in a transverse direction to join the small vein of Galen. The most anteriorly situated vein forms one of the tributaries of the small vein of Galen; the other two lie close together behind the lateral geniculate body and in front of the superior colliculus, joining the terminal part of the small vein of Galen. In addition to the intracerebral anastomoses mentioned above, the Galenic system is connected with the basilar veins by anastomoses which run across the lateral aspect of the midbrain (Fig. 2).

DISCUSSION

Abbie (1934), who has discussed the principles governing the distribution of the cerebral arteries, stressed primarily (among other points) the importance of functional constancy. "Whenever a neural mechanism evolves," he claims, "an artery develops for its supply and this neurovascular mechanism remains constant throughout evolution."

Our material, however, which furnishes evidence that arterial territories trespass across the boundaries of the nuclei in many instances, would seem to make it somewhat doubtful whether this principle can be generally applied. There is as yet no evidence that two or more vascular territories which participate in the supply of a single thalamic nucleus correspond to functional differences represented by the areas in question. The evidence gained from clinical observation, too, does not support the view of functional constancy, for in as many as 40% of human brains variations of the "normal" vascular arrangement have been described in the medulla oblongata (Foix *et al.* 1925) where the differentiation of function, as expressed by the highly intricate pattern of the nuclei and fibre tracts, is perhaps more obtrusive than in any other part of the central nervous system. In the arterial tree of the cerebral hemispheres, too, numerous variations are encountered. This fact was stressed particularly by Foix & Hillemand (1925) who studied the arterial supply of the temporal lobe, and by Moutier (1908) who made a very detailed analysis of the asymmetries in the vascular supply of the hemispheres and the variations of the Sylvian artery. Moutier was able to distinguish four types of ramifications of this vessel, which were present in 62, 24, 8 and 6% of brains respectively. Throughout the brain, variations of vascular syndromes, representing well-established clinical entities, can be readily explained by the variations in the territorial distribution of the arteries involved, and the figures quoted above could almost give rise to the assumption (in which there is probably some truth) that no more remains of the principle of constancy in arterial supply, within the confines of one species, than the natural limitations of variability allow. Even if this variability should be limited to the extracerebral course of the arteries (as maintained by Abbie) while the parenchymal end-arteries arising from the former adhere strictly to the vascular territories allotted to them throughout evolution, yet the fact that one and the same

end-artery may supply a complex of various nuclei and fibre tracts (which cannot be considered as a functional unit) would be an additional argument against the principle of functional constancy—though it does not exclude some sort of morphological constancy. In any case it is quite obvious that only comparatively small units, such for instance as the ansa lenticularis and the nuclei connected by it, could be supplied by one vessel, whereas this kind of vascularization would be impossible for systems of fibre tracts and their associated nuclei which extend, like the fronto-ponto-cerebellar tracts, throughout the brain, or, like the pyramidal tract, throughout the entire central nervous system.

There is therefore certainly no such thing as an "artery of a function", even in the modern human brain, although there is more than one well-known "artery of a syndrome", as, for instance, the left posterior temporal artery which is frequently referred to as "artère de l'aphasie de Wernicke", or the posterior inferior cerebellar artery, the occlusion of which causes Wallenberg's syndrome and its numerous variations.

It must be finally kept in mind, in regard to the question of functional constancy, that even well-defined structures which can be traced throughout the animal kingdom may have different functions, and that there is a general tendency recognizable during evolution for functions to "shift" towards the cerebral end of the neural axis as we ascend the phylogenetic scale.

In some cases, however, the constancy of arterial supply does seem to be maintained over a very wide range of this scale. For example, the anterior chorioidal artery has been found to participate in the supply of the anterior nucleus in man (Beevor, 1907; Foix & Hillemand, 1925). This is a very remarkable feature, as the course of this vessel along the optic tract and the lateral geniculate body on its way to reach the anterior nucleus is very long and may almost be regarded as a detour.[1]

In view of the difficulties involved in the assumption of any constant relationship between blood supply and function, it is worth while enquiring whether the intimate relationship between the anterior nucleus and the anterior chorioidal artery may be explained on a purely morphological basis. This discussion will serve to exemplify, at the same time, the general pattern of the arterial supply of the brain stem.

The division into a *dorsolateral* and a *ventromedial* group of vessels is obviously a general feature in the vascularization of the brain stem. The study of some instructive diagrams given in two of Böhne's papers (1927), in which is described the arterial supply of the medulla and the pons in man, suggests, when compared with the vascular territories of the spinal cord and our own findings, the following interpretation of the basic morphological pattern. The

[1] It may incidentally be considered as hardly compatible with the principle of convenience of source (which is also mentioned by Abbie) for, as described above, the inferior and middle anterior thalamic arteries arise at a very short distance from the anterior nucleus as compared with the origin of the anterior chorioidal.

most primitive conditions prevail in the spinal cord, where a ventral area, situated ventral to the central canal and supplied by the anterior spinal artery, can be distinguished from the dorsal area which is represented by the paired posterior spinal arteries. As the central canal opens to form the fourth ventricle, the two *dorsal* areas become displaced laterally and downwards on either side of the ventral area. They almost entirely lose their connexions with the two dorsal spinal arteries—which practically cease to exist—and receive their arterial blood from the posterior inferior cerebellar arteries. Only a very small field on either side of the midline belonging to the caudal part of the fourth ventricle and the adjacent parts of the restiform body remains for the terminal branch of the posterior spinal arteries and the posterior inferior branches of an artery termed by Böhne the posterior artery of the medulla oblongata. For the lower part of the medulla the division into two vascular territories, ventromedial and dorsolateral, becomes quite apparent. A diagram by Foix *et al.* (1925) demonstrates the wedge-shaped ventromedial and the two adjacent dorsolateral areas of the medulla still better, their converging boundaries running upwards towards the median fissure of the fourth ventricle without paying attention to the boundaries of nuclei. Thus the principles of arterial distribution in the medulla and the thalamus, two regions of the brain-stem far distant from each other, are identical, and are unquestionably linked up with Duret's concept of paramedian and circumferential arteries (1874).

This arrangement becomes somewhat modified in the regions between the medulla and the diencephalon as the cerebellum overlaps the pons and part of the midbrain, and blood from new dorsal sources, the superior cerebellar arteries, becomes available. Indeed, branches of this artery occupy a large part of the dorsolateral area of the pons and reach far downwards, as if there was in this region some sort of competition between the established dorsolateral vascular territories and the new dorsal territories. In the midbrain, too, a dorsolateral and a ventromedial vascular area exist.

On the basis of this principle, dividing transverse sections of the brain stem throughout into the two areas referred to, the long course of the anterior chorioidal artery may perhaps be understood more easily. It supplies, as the most anteriorly placed circumferential artery, the nervous tissue corresponding to the most anteriorly placed area of the dorsolateral thalamic surface.

Primarily there is no doubt some kind of relation between functional neurovascular units and purely morphological units. For in a primitive animal, or in an organism at an early stage of development, and even in higher organisms where the segmental arrangement is maintained in certain parts of the body, the relationship of arteries to functional units formed by any kind of tissue is still present. However, where the segmental architecture becomes changed, as a result of specific adaptation (and it may frequently become changed beyond recognition), a redistribution of the arteries takes place. As larger arteries develop in response to the establishment of more voluminous

masses of tissue, they tend to concentrate and to monopolize the distribution of blood, thus depriving subordinate areas of parts of their original vascular territories. This tendency to concentrate may explain the supply of parts of the pons by the large superior cerebellar arteries.

On the whole, the vascular territories in the remaining portions of the thalamus in man, as described by Foix & Hillemand (1925), are also similar to the areas described in the rabbit, if the difference in the shape of the thalamus and the differences in shape and relative size of the nuclei are taken into account. The vertical diameter of the "wedge" representing the ventromedial vascular area, however, appears to be reduced in man as compared with the rabbit, since in the former the dorsolateral territories approximate to and occupy the upper parts of the ventricular wall. An analogy exists in the form of an extension well towards the internal capsule of the "pédicule thalamo-perforé" or "pédicule retromammillaire", corresponding in its origin to the posterior inferior thalamic artery of the rabbit, but it resembles, as far as its distribution is concerned, the middle inferior thalamic artery. It is not possible at the moment to form an opinion on the possible significance of this feature, except that it may further confirm the tendency of the deep vascular area to send finger-like projections towards the internal capsule which is far distant from it. Softenings involving these territories have, therefore, a ribbon-like appearance in coronal sections, as illustrations given by Foix & Nicolesco (1926) and Nicolesco & Hornet (1934) show.

The last-named authors report common features encountered in two thalamic syndromes, the syndrome of the thalamo-geniculate (posterior superior thalamic) and the thalamo-perforate (posterior inferior thalamic) group of arteries. An occlusion of the former causes a hemianaesthesia on the opposite side of the body, the sensibility of the face remaining intact, while an occlusion of the thalamo-perforate vessels leads mainly to various ataxic disturbances. An obstruction of the posterior cerebral artery, immediately after it takes origin, gives rise to a combination of the two syndromes involving both groups of arteries. In these cases two separate areas of softening can be seen between which the centre median nucleus and the nucleus semilunaris (Flechsig) appear normal. Nicolesco & Hornet conclude, therefore, that these nuclei, on which they presume the sensibility of the face to depend, have a different arterial supply, but they are unable to identify a specific source. It will be remembered that during the descriptions of our specimens a gap in front of the posterior inferior thalamic artery was described which was occupied by the group of the middle superior thalamic arteries and which partly corresponds to the topographical position of the centre median nucleus or its equivalent in primates. As the middle superior thalamic vessels arise from the posterior communicating artery, which is independent of the vertebral artery, the sparing of the face area could be easily explained. The phylogenetic constancy of supply would in this case be less surprising, as the arrangement of the main group of vessels might conceivably be subjected to variations to a much smaller

degree than the neurovascular relationship of individual arteries within one group.

Finally, the lenticular or "lenticulo-optic" arteries may be briefly mentioned. Duret (1874), according to Foix & Hillemand, overestimates the role of these vessels in the vascularization of the human thalamus. In the rabbit none of these vessels could be traced to the thalamus.

The most primitive pattern of arterial supply of the neural tube is apparently represented by a system of small arteries which take origin from the vascular network surrounding the brain and radiate towards its central axis. This primitive type is largely maintained where the walls of the neural tube remain

Fig. 7. Transverse section through the thalamus of a rhesus monkey showing greater capillary density in the anterior and midline nuclei, as well as the obtrusive capillary pattern in the corpus subthalamicum and the zona incerta. Pickworth's stain.

comparatively thin, as, for instance, in the region of the anterior horn of the lateral ventricle (Fig. 6), but it can also be recognized in the arrangement of the arterioles in the remaining part of the cortex. The calibre of these vessels is small throughout as the cortex forms a comparatively thin layer, and the metabolism of the white substance underlying the cortex requires but a small amount of blood as compared with the grey matter where the larger part of the branches of the cortical arteries are given off. Where, however, extensive areas of grey matter establish themselves in the depth of the brain, the arterioles are replaced by large common trunks. The latter penetrate deep into the brain

substance, where, close to the buried surface of the grey matter and within its confines, a central tree of arterioles develops. Thus, while following the structures to be supplied, the small nutrient vessels give up their individual independence and become branches of larger arteries which have mainly the function of supply channels.

Within the dorsolateral arterial system the circumferential vessels act as supply channels. The distribution of the dorsolateral arterioles arising from the anterior, middle, and posterior superior thalamic arteries resembles to a certain extent the cortical pattern, as the grey matter lies close to the dorsolateral surface.

The finer configuration and the type of ramification of the vascular tree, quite apart from the general topographical arrangement within the two vascular areas, depend on the cytoarchitecture of the grey matter to be supplied. Thus, within a nucleus composed of layers of cells disposed parallel to its free surface, as, for instance, the lateral geniculate body of the rabbit, larger branches corresponding to these layers are quite a common feature—and the same holds true to a still greater extent for the layers of the colliculi and the cortex—whereas no such pattern can be seen where no regular laminar structure exists. The twigs of the arterial branch supplying the ventral nucleus, again, are arranged quite differently, but they, too, represent an example of the adjustment of an artery to its territory of supply.

Very little can be said about the influences governing the territorial distribution and the morphological features of the veins, except perhaps that some of the larger veins travel in the medullary laminae separating the various nuclei. The tendency of the veins to form intra- and extracerebral anastomoses is obvious. Arteriovenous anastomoses were seen in one or two instances. No precapillary anastomoses between arteries, however, could be detected.

The angioarchitecture of the capillary network shows a distinct relationship to the size and density of the ganglion cells, not only as far as the thalamus is concerned but also in most of the other areas of grey matter, for instance, the cortex. There are, however, exceptions to this rule, for Finley (1939) was able to show that, in the substantia nigra, certain areas which are composed of densely arranged cells are supplied by a comparatively scanty capillary network only. He maintains that specific metabolic requirements of the various areas play a determining part in their capillary architecture. Dunning & Wolff (1937) have expressed the opinion that there is some relationship between the number of synapses and the richness of the capillaries. Our material does not allow any conclusion as to the correctness of either of these assumptions, which are mentioned for the sake of completeness only.

SUMMARY AND CONCLUSIONS

1. The extrathalamic arteries in the rabbit may be divided into a dorsolateral and a ventromedial group.

2. Within the thalamus a ventromedial arterial territory, situated on either side of the median plane, can correspondingly be distinguished from a dorsolateral territory.

3. This vascular arrangement is encountered throughout the brain stem and is related to the existence of large supply channels at the *base* of the brain stem only. In the spinal cord, on the other hand, a ventral and a dorsal vascular area correspond to the ventral and dorsal spinal arteries.

4. The two adjacent ventromedial vascular areas in the thalamus may be asymmetrical, for the arteries on one side may trespass in the region of the midline nuclei far across the median plane.

5. The boundaries of the ventromedial and the dorsolateral arterial territories are highly irregular, for extensions of various different orders may protrude from the dorsolateral aspect far towards the median plane or from the ventromedial aspect towards the surface of the thalamus.

6. There is, however, some evidence to suggest that a number of these interlocking vascular extensions are, despite their apparent irregularity, a rather constant feature in the phylogenetic series and they probably explain some peculiarities of vascular diseases affecting the human thalamus.

7. There are no individual nuclear arteries in the thalamus of the rabbit, for the areas vascularized by the parenchymal arterioles—which must be considered as "end-arteries"—do not coincide with those of the nuclei or their subdivisions. Individual nuclei have a composite vascularization, and any one parenchymal arteriole supplies parts of adjacent nuclei and fibre tracts.

8. The lack of correspondence between individual nuclei and individual vascular territories is a very common feature in the brain, and the existence of arterial areas involving adjacent fragments of grey and white matter provides (among other reasons) no foundation for the theory of functional constancy which claims that functional units existing in the brain have arteries of their own throughout evolution, and that they may even be homologized on the basis of their arterial supply throughout a phylogenetic series.

9. An attempt is made to explain the fundamentally different arrangement of the arterioles in the dorsolateral and ventromedial areas of the thalamus.

10. The angioarchitecture of the thalamus is further complicated (*a*) by the usually different course of the arteries and veins, the territories of the latter, in sharp contrast to the arterial areas, overlapping each other to a great extent; (*b*) by morphological differences between arteries and veins, which are described in detail; (*c*) by the differences shown in the type of ramification of the arteries; and (*d*) by the varying density of the capillary network in the different nuclei.

11. It should be possible to predict to a certain extent the angioarchitecture in a given area as some of the factors determining these differences can be analysed.

12. The extrathalamic veins form widespread anastomoses. Venous anastomoses are also encountered within the thalamus, where they chiefly lie near the walls of the third ventricle on either side of the median plane. They connect the basal veins with the veins of Galen.

I wish to express my thanks to Prof. Le Gros Clark at whose suggestion this investigation was undertaken, and who provided the facilities for it. The material showing the vascular architecture in the macaque thalamus (to which reference is made in the discussion) was prepared by him and placed at my disposal. I gratefully acknowledge the assistance of a grant from the Nuffield Committee for the Advancement of Medicine.

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THE NORMAL HISTOLOGY OF THE THALAMUS IN THE PHALANGER, *TRICHOSURUS VULPECULA*

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In recent years the thalamus in several orders of placental mammals has been studied, using both normal histological material and experimental methods. Knowledge of the main cytoarchitectural features and connexions of the different thalamic nuclei has been established on a firm basis, a consistent nomenclature has been developed, and a fundamental pattern has been described from which the condition in more advanced mammals, such as the higher primates, can be derived.

Of the marsupial mammals, only the relatively primitive polyprotodont, *Didelphys virginiana*, has been described in detail (Tsai, 1925; Chu, 1932*a*, *b*; Bodian, 1935, 1937, 1939, 1940). It seemed therefore desirable that an investigation of a more advanced diprotodont marsupial should be undertaken, the more so since an examination of the motor cortex and its connexions in such an animal (Goldby, 1939) had already shown important differences from the condition in the opossum. Work done on other mammals has emphasized the necessity for experimental methods if any precise information about thalamic connexions is to be obtained; such methods can be applied only on the basis of a knowledge of the normal histology of the thalamus, and it is the purpose of this paper to supply such a basis.

MATERIAL AND METHODS

The animals used were all adult specimens of the phalanger, *Trichosurus vulpecula*, and the description which follows is based principally on two complete series of sections: a transverse series cut at 40 μ and a sagittal series cut at 30 μ, both in celloidin. In both series alternate sections were stained by toluidine blue and by Weil's modification of the Weigert method. Several additional transverse series were used, cut from brains in which cortical lesions had been made about 3 months before the animal's death. The lesions were all unilateral, so that one side only was affected by them. For the most part they were cut at 40 μ in celloidin, and alternate sections mounted and stained by toluidine blue. Usually every eighth section was stained by Weil's method.

Two series were available, both transverse, cut in paraffin at 12 μ; alternate slides were stained with toluidine blue and Davenport's reduced silver method. Two other paraffin series stained with cresyl violet (transverse and sagittal) were prepared but were found to give no information which could not be obtained more readily from the thicker celloidin sections.

It is obvious that this material, though adequate for a description of the principal cell masses and fibre tracts of the thalamus, cannot give detailed information about its cytology or the connexions of its fibres. No fibre can be traced to its precise origin or termination, and in many cases it is impossible to distinguish between fibres of passage and fibres which begin or end in the region under examination. For these reasons more attention has been given to a description of the cell masses and the arrangement of fibres so far as these help to differentiate the cell masses, than to actual fibre connexions. In most cases, probably, the apparent ending of a fibre tract in a cell mass, or its intimate relationship to one, should be interpreted as an actual connexion; the evidence is of contiguity only however, and often, notably in the optic system, conclusions based on it have been found to be unreliable when tested by experimental methods. Some evidence of this kind can be found in the material used here, for most of the connexions that have been described, from similar evidence, in other primitive mammals. Because of its inconclusive nature it has not been thought necessary to report it all in detail.

In general the present material is similar to that used by most workers in this field. The results obtained should therefore be comparable with those of others; it is inevitable, however, that the personal factor should influence the decision when it is a question of what degree of differentiation is necessary before a particular mass of cells or fibres should be called a nucleus or tract and given a specific name. This is especially so with such nuclei as those of the midline, or the subdivisions of the principal nuclei. Apparently minor differences of technique, e.g. the use of thick or thin, celloidin or paraffin sections, may alter appearances considerably, and sometimes slight differences in the plane of section may have a similar effect. These difficulties must be carefully considered in making comparisons based on the examination of material of the kind used for this paper.

The classification of nuclei and the terminology which has been adopted is based mainly on the work of Clark (1932, and in other communications); some modifications have been introduced, partly for convenience of description in this particular animal, and partly to bring the work into line with that of Bodian on the opossum. In so far as the results justify phylogenetic or functional conclusions, they will be dealt with briefly in the discussion; such problems will need the more precise information which can be gained from experimental methods for their full elucidation.

GROSS ANATOMY OF THE THALAMUS

(Text-figs. 1, 2)

The thalamus of the phalanger consists of an obliquely placed ovoid mass of grey matter above and medial to the cerebral peduncle and the internal capsule, and anterior to the superior corpora quadrigemina (Text-figs. 1, 2). In general appearance it is very like that of most primitive mammals.

Its lateral surface, except where it is obscured by the internal capsule, is formed mainly by the lateral geniculate body, which overlaps posteriorly the prominent medial geniculate body. The latter has the usual relationship to the inferior brachium and to other midbrain structures. Optic tract fibres sweep over the lateral geniculate body and the posterior half of the dorsal surface of the thalamus, apparently going to the superior corpus quadrigeminum; at least the anterior part of the medial geniculate body is similarly covered. Many of these fibres are probably not of retinal origin, as Bodian (1937) has shown in the opossum. The posterior accessory optic tract (tractus peduncularis transversus) is not visible on the surface of the peduncle as in many mammals.

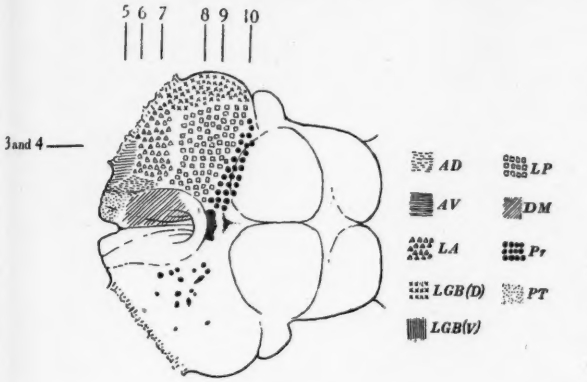


Fig. 1.

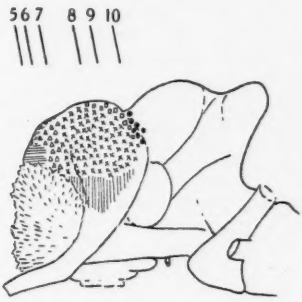


Fig. 2.

Text-fig. 1. A drawing of the dorsal aspect of the thalamus and midbrain of *Trichosurus vulpecula*, × 2·5. The lines marked 3–10 indicate the levels of the sections illustrated in the corresponding figures. In the left thalamus a number of vascular foramina are shown; in the right the position of some of the thalamic nuclei has been marked.

Text-fig. 2. A drawing of the lateral aspect of the thalamus and midbrain of *Trichosurus vulpecula*, × 2·5. The lines marked 5–10 have the same meaning as in the previous figure, and the same conventional markings have been used for the nuclei.

The dorsal aspect differs considerably from that of the opossum as illustrated by Tsai (1925). It is much broader posteriorly, an effect of the greater development of the posterior part of the lateral nuclear complex. There is a depression on this surface, not very well defined, lateral to the posterior part of the stria medullaris, which is penetrated by many fairly large blood vessels (Text-fig. 1, left side). It marks approximately the region of transition from the main anterior to the main posterior parts of the lateral complex. The pineal body is small; it lies as usual between the habenular and posterior commissures, but is scarcely raised above the surface. The rest of the epithalamus calls for no special comment.

Practically the whole of the medial surface is occupied by a large massa intermedia; the anterolateral and ventral surfaces are bounded by an external

medullary lamina which has the usual relationships to the internal capsule and the subthalamus.

In Text-figs. 1 and 2 the position of some of the thalamic nuclei is shown, projected on the dorsal and lateral surfaces. The anteroventral nucleus appears smaller than it really is, since the slope of the anterior part of the thalamus causes considerable foreshortening, and in the lateral view it is partly concealed by the internal capsule.

In gross features the main difference from the opossum consists in the greater breadth of the posterior part of the thalamus, and in the slightly greater size of the lateral geniculate body. In the midbrain the superior corpus quadrigeminum is definitely larger than the inferior, while the opposite seems to be true in the opossum (Tsai, 1925). All these features suggest a greater development of structures related to the optic tract, or of the visual system in general. Although as a whole the shape is rather different, there are many resemblances to the thalamus of *Tupaia* (Clark, 1929*a*) where, however, the size of the optic tract and superior corpus quadrigeminum is relatively still greater. No pulvinar, visible on external examination, is present in the phalanger as there is in *Microcebus* (Clark, 1931).

It may be said that in external appearance the thalamus of the phalanger differs from that of the opossum in much the same way (but to a smaller degree) that the thalamus of more advanced placental mammals differs from the more primitive. This difference is largely due to growth of the posterior parts of the thalamus which are related to the visual system.

INTERNAL STRUCTURE OF THE THALAMUS

The thalamus as a whole is enclosed ventrally and laterally by an external medullary lamina, and is divided by an internal medullary lamina into a smaller dorsomedial and a larger ventrolateral part. In this description the anterior group of nuclei will be included in the ventrolateral subdivision. They are separated from the parataenial and paraventricular nuclei (dorsomedially situated) by an anterior extension of the internal medullary lamina in which is found the commissure and commissural nucleus of the anterodorsal nucleus. In higher mammals (e.g. Carnivora, Rioch, 1929; the macaque, Aronson & Papez, 1934) the internal medullary lamina and its nuclei appear to extend forwards below the whole anterior group of nuclei, separating them from the anterior part of the ventral nucleus. The close relationship between the nucleus commissuralis interanterodorsalis and the nucleus centralis lateralis was stressed by Clark (1930) for *Tarsius* and by Bodian (1939) for the opossum. The nucleus centralis lateralis is a nucleus of the internal medullary lamina, and its displacement ventral to the whole anterior group of nuclei and away from the nucleus of the anterodorsal commissure in higher mammals would appear to be secondary, perhaps a result of the relative decrease in size of the anterior nuclei and the growth forward beneath them of the ventral nucleus.

The two sides are separated in the median plane of the massa intermedia by a number of midline and commissural nuclei. These nuclei contain a network of fine medullated and non-medullated fibres connected with the internal and external medullary laminae and with the periventricular system. Some are commissural, but no predominance of transversely directed fibres was found in any part of the median plane. Commissural connexions between the two sides appear to be far less well developed in the phalanger than in the opossum (Bodian, 1940); it may be that failure in staining or silver impregnation is the reason for this finding, but such a failure was not obvious in any other parts of the sections.

Since the medullary laminae and their nuclei (including those of the midline) form a scaffolding in which the principal thalamic nuclei are set, it will be convenient for descriptive purposes to deal with them first. The nuclei are for the most part poorly defined from each other and from the principal nuclei, so that some of the differences between this and other descriptions will be of doubtful significance.

Nucleus centralis (Pl. 1, figs. 5–7)

This nucleus consists of a fairly compact mass of cells running anteroposteriorly in the massa intermedia at the level of the internal medullary lamina. It begins anteriorly (Pl. 1, fig. 5) between the parataenial and paraventricular nuclei above, and the nucleus reuniens below, and gradually disappears posteriorly about the level of the habenular commissure. It is made up of small cells, rounded or spindle-shaped, set in a dense feltwork of fine myelinated fibres mainly derived from the internal medullary lamina. This feltwork appears to receive contributions from the periventricular system both anteriorly and posteriorly.

In transverse section the nucleus is characteristically diamond-shaped (Pl. 1, fig. 7). The lateral angles extend between the dorsomedial and ventral nuclei into continuity with the paracentral nuclei, but anteriorly between the parataenial and paraventricular nuclei above and the anteromedial and anteroventral nuclei below. This anterior part of the lateral extension from the central nucleus forms the anterodorsal commissural nucleus. The dorsal angle is related to a median seam of cells which lies between the paraventricular and parataenial nuclei anteriorly and between the dorsomedial nuclei posteriorly. In this seam a number of commissural nuclei are developed. The ventral angle is related to the nucleus reuniens but is separated from it posteriorly by a fusion between the two ventral nuclei. A small collection of cells probably corresponding to Bodian's nucleus commissuralis interventralis anterior can be distinguished, but is poorly defined and variable; it hardly merits a separate name.

The rhomboid nucleus described by Bodian and other authors has been included in the central nucleus as described here, and is represented by the

anterior and dorsal part of that nucleus. There seemed no adequate justification in this material for describing it separately. Commissural fibres, though present, are not conspicuous; otherwise the nucleus centralis of the phalanger does not appear to differ significantly from the same nucleus in other mammals.

The median raphe (Pl. 1, figs. 5–8)

Anteriorly, between the anterior paraventricular nuclei, there is a well-defined median seam of cells. Dorsally, near the ependyma, the cells are small, deeply stained and polygonal; ventrally they are slightly larger, paler, and spindle-shaped. These more ventral cells lie between the ventral parts of the parataenial nuclei; few if any commissural fibres could be seen among them. They are closely related to the anterior periventricular fibres.

A little posteriorly, just before the dorsomedial nuclei appear, this seam of cells becomes more extensive and less clearly defined. It blends below with the nucleus centralis and laterally with the anterior paraventricular nuclei and with some irregular clumps of small cells (Pl. 1, fig. 5) among the inferior and intermediate thalamic radiations which appear to be going mainly to the dorsomedial nucleus. These radiations are particularly abundant here and probably correspond with Rioch's anterior thalamic radiation (1931). The clumps of cells among them cannot be clearly defined from the commissural part of the anterodorsal nucleus.

Soon after the dorsomedial nuclei have appeared in a caudal direction the median seam of cells becomes inconspicuous, except for a small clump which may represent a commissural nucleus. For the most part it blends with the paraventricular nuclei to form a triangular mass of cells, dorsally situated beneath the ependyma and between the habenular nuclei (Pl. 1, fig. 8). This triangular mass is the beginning of the posterior paraventricular nucleus of most authors.

Paraventricular nuclei (Pl. 1, figs. 5–8; Pl. 2, fig. 9)

These nuclei are usually described in two parts, anterior and posterior, continuous with one another along the dorsal surface of the massa intermedia. The anterior paraventricular nuclei are well developed in the phalanger; they appear in the most anterior part of the massa intermedia as comma-shaped masses of small cells on either side of the median seam bounded laterally by the parataenial nuclei. In most descriptions the cells of the median seam have been included in the paraventricular nuclei; at least in the anterior part of the thalamus they are distinct in the phalanger. Passing backwards this distinction is practically lost at about the level of the middle of the dorsomedial nuclei. The nuclei of the two sides blend to form the triangular mass of cells already mentioned, although the cells actually in the median plane remain different from the rest, being slightly smaller and more deeply stained.

This triangular mass of cells is probably the beginning of the nucleus paraventricularis posterior described in the opossum (Bodian, 1939) and other mammals. It extends posteriorly above, below, and among the fibres of the posterior commissure (Pl. 2, fig. 9). It spreads ventrally to occupy the whole posterior border of the massa intermedia (where it blends with the medial part of the parafascicular nucleus) and laterally between the fasciculus retroflexus and the posterior commissure to reach the pretectal nucleus. It blends posteriorly with the central grey matter of the midbrain. It is continuous, below the parafascicular nucleus, with the nucleus subparafascicularis (Pl. 2, fig. 9).

Both anterior and posterior paraventricular nuclei are related intimately with the anterior and posterior parts of the periventricular fibre system. This is particularly so with the posterior nucleus, which might be described as an interstitial nucleus of that system. By this means the nuclei are probably linked with the hypothalamus and perhaps with the midbrain through the dorsal longitudinal bundle of Schütz. In addition the posterior paraventricular nucleus appears to have strong connexions with the posterior commissure. Bodian (1940) has described connexions between both nuclei and practically all the adjacent thalamic nuclei and the tectum; it is probable that similar connexions are present in the phalanger.

Nucleus paracentralis and Nucleus centralis lateralis (Pl. 1, figs. 6, 7)

The paracentral nucleus consists of cells that extend laterally from the nucleus centralis into the internal medullary lamina; they are of medium size and larger than those of the nucleus centralis. It is nowhere clearly defined from the dorsomedial nucleus above or the ventral nucleus below, and is especially diffuse posteriorly. It is coextensive with the ventral surface of the dorsomedial nucleus, giving place anteriorly to the irregular clumps of cells found between the dorsomedial and the parataenial nucleus, and to the commissural part of the anterodorsal nucleus; here it is indistinguishable from the nucleus centralis lateralis. Posteriorly it becomes more and more difficult to distinguish from the dorsomedial nucleus, and is finally replaced by the parafascicular nucleus.

In the part of the internal medullary lamina which turns dorsally towards the surface of the thalamus is found a similar group of cells (Pl. 1, fig. 6) which lacks clear definition and merges with the lateral part of the dorsomedial nucleus. This is the lateral central nucleus, and as with the paracentral it is mainly the presence of the fibres of the internal medullary lamina which serves to distinguish it from neighbouring nuclei. It disappears posteriorly in front of the upper end of the fasciculus retroflexus; anteriorly it seems to become the commissural part of the anterodorsal nucleus.

Both these nuclei are presumably connected with the fibres of the internal medullary lamina. Through it they may be brought into relationship with

adjacent nuclei, the nuclei of the midline, the periventricular system and the thalamus of the opposite side. Both are penetrated by thalamic radiations, but these are probably fibres of passage on their way to or from the dorsomedial nuclei.

Nucleus reuniens (Pl. 1, figs. 5–7)

The nucleus reuniens of this description includes the nucleus subparataenialis and the nucleus reuniens anterior and posterior described by Bodian (1939) in the opossum. It corresponds therefore with the nucleus reuniens of Rioch (1929) in Carnivora and of Gurdjian (1927) in the rat. The three nuclei described by Bodian were recognizable as topographical regions, which did not seem to be differentiated clearly enough to justify description as separate nuclei.

The nucleus reuniens begins in the most anterior part of the thalamus as a mass of cells ventral to the parataenial nucleus from which it is not clearly defined; it is soon separated by the appearance of the commissural part of the anterodorsal nucleus. In this part of the thalamus it is separated from its fellow by a thin but nearly cell-free median raphe; laterally it is related to the anteromedial nucleus. Its cells are of medium size, but larger dorsolaterally than ventromedially. The dorsolateral part corresponds to Bodian's nucleus subparataenialis. Posteriorly it comes to lie between the ventral nuclei; here it is smaller, and fuses across the midline with its fellow (this fusion is already beginning in Pl. 1, fig. 5). The larger cells which it contained anteriorly gradually disappear; it loses definition and ends in a tail of small cells which runs back in the ventral border of the massa intermedia to the posterior paraventricular nucleus (Pl. 1, fig. 7).

The chief connexions of the nucleus reuniens appear to be with the anterior periventricular system among the fibres of which its cells are disposed. These fibres sweep from the dorsomedial part of the thalamus, through the nucleus parataenialis and the anterior paraventricular nucleus in the anterior border of the massa intermedia, into the nucleus reuniens. They continue ventrally to the hypothalamus, but a few of the most medial fibres pass backwards into the posterior periventricular system. The common relationship of these nuclei to the periventricular system suggests some community of function, perhaps as interstitial nuclei in the pathway between the dorsomedial nucleus and the hypothalamus. Some fibres from the inferior thalamic radiations run into the dorsolateral part of the nucleus anteriorly; these do not appear to be so abundant as the similar connexions described by Bodian (1940) in the opossum for the nucleus subparataenialis. Commissural fibres associated with the nucleus reuniens are scanty in the phalanger, but there are probably connexions with the nucleus centralis and the adjacent anteromedial and ventral nuclei.

Posteriorly the nucleus reuniens joins the paraventricular nucleus where it extends laterally ventral to the nucleus parafascicularis as far as the medial geniculate body (Pl. 2, figs. 9, 10). This lateral extension is the nucleus sub-

parafascicularis (Bodian, 1939), and it is accompanied by a bundle of fine fibres which connect medially with the posterior paraventricular nucleus and the nucleus reuniens. This is the tractus tecto-reuniens described by Bodian (1940); in the phalanger it is not well developed and most of its fibres appear to end laterally in the posterior part of the ventral nucleus. A few can be traced to the medial geniculate body, but no other connexions could be determined from the material available. There is no well-developed interventral commissure in the phalanger, and none of the fibres of the tractus tecto-reuniens could be followed across the midline.

It seems probable that the nucleus reuniens of the phalanger is relatively a little smaller and less clearly differentiated than in the opossum, but better developed than in most mammals. From Clark's description it is not a very conspicuous feature of the thalamus either in *Tupaia* (1929*a*) or *Tarsius* (1930), but without first-hand knowledge of the material it is doubtful if these comparisons have much value. It is noteworthy that the nucleus reuniens of the macaque, as described by Aronson & Papez (1934), is very like that of the phalanger, while Walker (1938), in another account of the macaque thalamus, considered it too ill-defined to justify description as a separate nucleus.

Nucleus parafascicularis (Pl. 2, fig. 9)

This is a well-developed nucleus and is more clearly defined than any mentioned so far. It lies in the posterior part of the internal medullary lamina where it clothes the posterolateral surface of the dorsomedial nucleus. The fasciculus retroflexus divides it into two parts, medial and lateral, which are joined by a thin layer of cells on the anterior surface of the fasciculus. Posteriorly they remain distinct. Both parts lie posterior to the dorsomedial nucleus, from which they are quite clearly defined.

The cells of the nucleus as a whole are conspicuous from their deep staining; they are mostly of medium size, but those of the medial part are smaller and more closely packed than those of the lateral.

The medial part is related medially and posteriorly to the posterior paraventricular nucleus which separates it from the fibres of the posterior commissure; its boundaries are not sharp. The lateral, and much the larger part, lies posterior to the dorsomedial, paracentral and lateral central nuclei. In this region the only feature which distinguishes the paracentral and lateral central nuclei from the dorsomedial nucleus is the presence between their cells of the fibres of the internal medullary lamina. Posterodorsal to the lateral part is the pretectal nucleus; lateral to it the hinder end of nucleus lateralis B, and the ventral nucleus. Both parts lie above the subparafascicular nucleus. The lateral part extends posteriorly considerably further than the medial, but disappears from transverse sections as the fasciculus retroflexus enters the tegmentum.

The connexions of the parafascicular nucleus are mostly of a diffuse nature and difficult to define accurately in the material available. It contains a very

dense feltwork of fibres of the internal medullary lamina through which it is probably connected with most or all of the adjacent nuclei. Its medial part is particularly closely associated with the posterior periventricular system, and a few of the thalamic radiations enter it ventrally and laterally. Connexions with the tectum are probably present.

No representative of the centre median nucleus of primates is present in the phalanger except the lateral part of the nucleus parafascicularis from which it is probably differentiated in higher mammals. In other respects the parafascicular nucleus of the phalanger does not show any significant peculiarities.

Nucleus subparafascicularis (Pl. 2, figs. 9, 10)

This nucleus is clearly present in the phalanger in the same form as in the opossum (Bodian, 1939). It appears anteriorly as a lateral extension of the posterior paraventricular nucleus ventral to the nucleus parafascicularis. Its cells are larger than the paraventricular cells, and fusiform in shape; they do not stain deeply. It can be followed laterally to the ventral surface of the medial geniculate body, and is accompanied by a tract of fibres, the tractus tecto-reuniens (see p. 205), most of which are non-myelinated so that it forms a conspicuous pale area in sections stained by Weigert's method. Possible homologies for this nucleus and tract have been discussed by Bodian (1939, 1940); they call for no further comment here.

THE DORSOMEDIAL SUBDIVISION OF THE THALAMUS

Nucleus parataenialis (Pl. 1, fig. 5)

This nucleus is found in the most anterior part of the thalamus ventromedial to the stria medullaris. It follows the stria medullaris posteriorly until it is replaced by the dorsomedial nucleus. It consists of medium to large cells which do not stain very deeply with toluidine blue.

The parataenial nucleus reaches ventrally in the anterior pole of the thalamus, medial to the stria medullaris, down to the anterior end of the nucleus reuniens, from which it can be distinguished only with difficulty. The apparent continuity is soon broken by the anterodorsal commissure and its nucleus. The parataenial nucleus now lies between the paraventricular nucleus medially and the stria medullaris and the anterodorsal nucleus laterally; ventrally it is separated from the nucleus reuniens and the anteromedial and anteroventral nuclei by the anterodorsal commissure. As was pointed out before, the latter seems to represent the most anterior part of the internal medullary lamina. The parataenial nucleus becomes rapidly smaller as it is traced posteriorly and ends in the dorsolateral part of the nucleus dorsomedialis.

The most obvious connexion of the parataenial nucleus in this material is with the anterior periventricular fibres (see description of nucleus reuniens).

It has few commissural fibres, but is connected with its fellow by a strand of cells above the anterior part of the nucleus centralis. Some of the inferior and perhaps of the superior thalamic radiations reach it as Bodian described in the opossum; the origin or destination of these fibres must await experimental investigation. Connexions with all adjacent nuclei are probably present.

The nucleus parataenialis of the phalanger corresponds very closely with that of the opossum (Bodian, 1939) and of other mammals. Such differences as have been observed are matters of detail and of doubtful significance.

Nucleus dorsomedialis (Pl. 1, figs. 6–8)

This nucleus is well developed and occupies most of the region dorsomedial to the internal medullary lamina, which, with the intralaminar nuclei, separates it from the ventrolateral complex. Anteriorly it is replaced by the nucleus parataenialis, and posterolaterally by the fasciculus retroflexus and the parafascicular nucleus. Dorsally it is covered by the stria medullaris and by the medial and lateral habenular nuclei, and medially the paraventricular nucleus and the seam of cells in the median plane are found. The latter is conspicuous anteriorly (Pl. 1, fig. 7), but posteriorly the two dorsomedial nuclei fuse almost indistinguishably above the nucleus centralis (Pl. 1, fig. 8).

Except posterolaterally, where Meynert's bundle and its surrounding nucleus make a precise boundary, the dorsomedial nucleus is nowhere clearly defined from adjacent nuclei. Anteriorly it blends with a tail of cells from the parataenial nucleus, but is separated from the main part of that nucleus by the irregular clumps of small cells already mentioned (see description of median raphe and Pl. 1, fig. 5). Both laterally and ventrally there is a gradual transition to the lateral central and paracentral nuclei respectively, and the same applies to the midline nuclei on the medial side. There are in fact three subdivisions of the dorsomedial nucleus, defined mainly by their relationship to the intralaminar and midline nuclei (Pl. 1, fig. 7): the principal part, dorsomedially situated and composed of medium to large cells, evenly spaced; a dorsolateral part of smaller more closely packed cells, blending with the nucleus centralis lateralis; and a ventral part of larger cells which stains more deeply, and which blends with the paracentral nucleus. The dorsolateral small-celled part is conspicuous in the slides stained by the Weigert method for the few fibres it contains. The other two parts contain a well-developed network of fine myelinated fibres.

The dorsomedial nucleus receives many of the inferior and intermediate thalamic radiations throughout its length; these, after piercing the internal medullary lamina, appear to end chiefly in the dorsomedial and ventral parts of the nucleus. It is closely related to the anterior and posterior periventricular fibres and is probably connected through the internal medullary lamina with adjacent nuclei and with the opposite side. Few if any direct commissural fibres between the two nuclei could be seen.

In general the dorsomedial nucleus shows no striking differences from the same nucleus as described in other mammals. Its subdivisions do not appear to correspond with those described by Chu (1932*a*) for the opossum, but Bodian (1939), in the same animal, does not appear to recognize any subdivisions. In its relationship to the parataenial nucleus it differs slightly from the opossum; in the latter the parataenial nucleus extends posteriorly on its medial side (Bodian, 1939) as also occurs in Carnivora (Rioch, 1929) and in the rat (Gurdjian, 1927). In this particular respect the phalanger resembles *Tupaia* (Clark, 1929*a*), but as boundaries are difficult to define in this region, no great importance can be attributed to the observation. It is perhaps more significant that the posterior boundary between the dorsomedial nucleus and the parafascicular nucleus is more precise in the phalanger than appears to be the case in the opossum (Bodian, 1939).

The most definite difference from the opossum lies in the appearance of a dorsolateral small-celled part. Topographically this part corresponds with a similar region in *Tupaia* and *Tarsius* (Clark, 1929*a*, 1930) and with the better developed dorsolateral part of the nucleus in higher primates (Clark, 1930). In the macaque, Walker (1938) describes this region as containing fewer fibres than the rest of the dorsomedial nucleus, as is the case in the phalanger. It may represent the main part of the nucleus mediodorsalis of Rioch (1929) in Carnivora, and if so is a constant element in the thalamus of all but the most primitive mammals. It is present in the armadillo (Papez, 1932, pars dorsalis of the medial nucleus), which in respect of the dorsomedial nucleus as a whole seems very similar to the phalanger. It may be added that no representative of the nuclei of the tractus habenulo-peduncularis, as described by Rioch and others in Carnivora, was found in the phalanger.

THE VENTROLATERAL SUBDIVISION OF THE THALAMUS

As was said earlier the anterior nuclei belong, at least topographically, to this part of the thalamus. They are well developed in the phalanger, and have the usual subdivisions clearly defined from each other.

Nucleus anterodorsalis (Pl. 1, figs. 5, 6)

Though the smallest of the anterior nuclei, the anterodorsal nucleus is conspicuous on account of its characteristic large, deeply staining and closely packed cells. It appears in the most anterior part of the thalamus partly among and partly lateral to the fibres of the stria medullaris. It is dorsomedial to the anteroventral nucleus and lateral to the parataenial nucleus. It runs posteriorly for a considerable distance (Text-fig. 1), always maintaining a close relationship to the stria medullaris, and ends about a third of the way towards the posterior end of the thalamus and on its dorsal surface. In its posterior part the nucleus lateralis A is ventral to it (Pl. 1, fig. 6), and it marks approximately the dorsolateral extremity of the internal medullary lamina.

A very well-marked commissural nucleus is associated with the anterior part of the anterodorsal nucleus. It consists of small fusiform cells, closely packed, lying between the parataenial nucleus (dorsomedially) and the anteroventral and anteromedial nuclei (ventrolaterally), and it joins the anterior part of the nucleus centralis (the nucleus is seen in Pl. 1, fig. 5, although continuity with the nucleus centralis is not visible in this section). It lies in what appears to be an anterior continuation of the internal medullary lamina, but instead of the network of fibres which is characteristic of the greater part of the lamina, it contains in this region fibres which run predominantly towards the median plane forming the commissure of the anterodorsal nuclei. Posteriorly this commissural nucleus passes without any defined boundary into the paracentral and lateral central nuclei.

The anteroventral and anteromedial nuclei (Pl. 1, figs. 5, 6; Text-figs. 3, 4)

These nuclei form most of the ventrolateral subdivision of the thalamus in its anterior quarter; posteriorly they give place to the lateral and ventral nuclei.

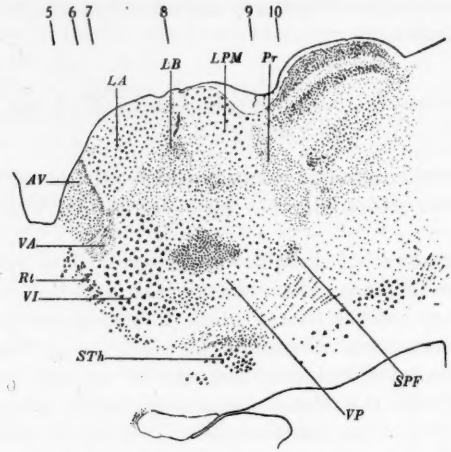
The anteroventral nucleus is slightly the larger; it consists of cells which are smaller than those of the anteromedial nucleus, and stain less deeply. Its boundaries are very definite except where it borders on the anteromedial nucleus; it is separated from the latter posteriorly by the termination of the mammillo-thalamic tract, but elsewhere can be distinguished only by the difference in size of cell. It is related dorsomedially to the anterodorsal nucleus, until it is separated by the nucleus lateralis A (Pl. 1, fig. 6). Nucleus lateralis A also forms most of its posterior boundary (Text-fig. 3), and the anterior part of the ventral nucleus is posteromedial to it. Ventrolaterally it is related to the internal capsule and the nucleus reticularis.

The anteromedial nucleus lies between the anteroventral nucleus and the nucleus reuniens, resting on the external medullary lamina. It extends rather further posteriorly than the anteroventral nucleus, but is finally replaced by the most medial part of the ventral complex. This posterior part of the anteromedial nucleus is distinguished by the close and regular arrangement of its cells, which lie near to the midline between the nucleus centralis and the nucleus reuniens. In the transverse series these closely packed cells have the appearance of a separate nucleus, but in the sagittal series they appear as the most posterior part of the anteromedial nucleus.

The most characteristic connexion of the anterior nuclear group is the mammillo-thalamic tract, which enters from behind between the anteroventral and anteromedial nuclei. Fibres of this tract end in both nuclei, probably more in the anteroventral than in the anteromedial. None could be traced to the anterodorsal nucleus. Traced in the other direction the tract runs along the lateral border of the anteromedial nucleus until that nucleus disappears; it

continues posteriorly in a depression in the most medial part of the ventral complex, finally turning down to the mamillary body. It does not pierce a part of the ventral nucleus as it does in some animals (e.g. in Carnivora, Rioch, 1929).

All three anterior nuclei receive fibres from the intermediate and superior thalamic radiations. These appear to be rather more abundant in the anteroventral nucleus than in the others. The anterodorsal nucleus has fairly strong commissural connexions, and is probably linked to the nuclei of the midline; its very close association with the stria medullaris (some of the cells form an



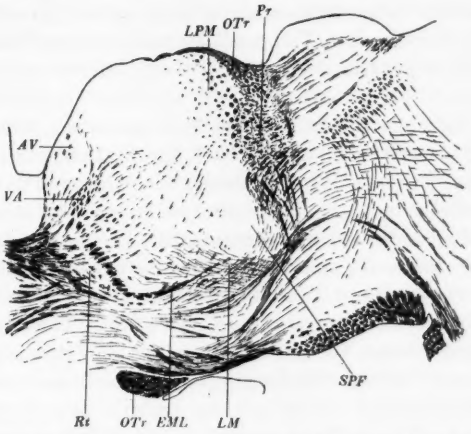
Text-fig. 3. A parasagittal section of the thalamus and midbrain of *Trichosurus vulpecula*, showing the disposition of the cell groups; × 6. Semidiagrammatic. The plane of this section is indicated in Text-fig. 1. The lines marked 5–10 have the same meaning as in the previous figures.

interstitial nucleus among its fibres) suggest a functional relationship with this system, although no conclusive evidence for this was found. The three anterior nuclei are probably connected with each other and with adjacent thalamic nuclei.

In general the anterior nuclei of the phalanger are very like those of the opossum (Bodian, 1939, 1940). As a minor difference it may be noted that the commissure of the anterodorsal nucleus is more closely related to the anterior than to the posterior part of that nucleus; also that the anteroventral and anteromedial nuclei can be differentiated on the basis of cell type in the phalanger but not in the opossum.

A similar difference in cell type between the anteroventral and anteromedial nuclei was noted by Clark (1929*b*) in *Erinaceus*, which, together with the poor development of the anterodorsal nucleus, he regarded as a primitive

feature. There is, however, a considerable degree of variation in this anterior group of nuclei among mammals generally considered primitive; in the armadillo (Papez, 1932), though in most respects the thalamus is of a very generalized type, there is no distinction between the anteroventral and anteromedial nuclei, and the anterodorsal nucleus is well developed; marked variation was noted by Clark (1929*b*) among the Insectivora. For these reasons the slight differences observed between the phalanger and other primitive mammals seem of doubtful significance; one can say, however, that as a whole the anterior nuclei show none of that relative diminution in size and degree of differentiation characteristic of Carnivora and of the higher primates.



Text-fig. 4. A parasagittal section immediately adjacent to the one shown in Text-fig. 3, stained by Weil's method to show the disposition of myelinated fibres.

Nucleus lateralis (Text-figs. 3, 4; Pl. 1, figs. 6–8; Pl. 2, fig. 9)

The nucleus lateralis forms the whole dorsal surface of the thalamus between the stria medullaris medially and the lateral geniculate body laterally, except for a small area occupied by the anteroventral and anterodorsal nuclei (Text-fig. 1). Posterior to the habenular commissure its medial boundary is the pretectal nucleus which separates it from the superior corpus quadrigeminum. Laterally it is bounded by the external medullary lamina which intervenes between it and the reticular nucleus (anteriorly) and the lateral geniculate body (posteriorly). Ventrally it rests on the ventral nucleus from which it is never very clearly defined.

It presents three subdivisions, which, because they appear to correspond with the subdivisions described by Clark (1929*a*) in *Tupaia* and by Clark and others in several different mammals, have been named similarly: nucleus lateralis A and B, and nucleus lateralis posterior. It may be said at once that

the subdivisions A and B have been taken to correspond with Bodian's pars intermedia in the opossum. His pars anterior seems to be present in the phalanger, where it is represented by a small collection of cells on the dorso-lateral surface of the thalamus anterior to the lateral geniculate body. These cells can be seen (though not very clearly) in Pl. 1, fig. 7. This collection of cells is very variable; sometimes it raises a small elevation on the surface, but in some specimens it is almost completely absent. The cells are slightly smaller than those of the nucleus lateralis A. There is no network of fibres among them as there is in the adjacent lateral geniculate body. They are covered superficially by fibres of the stratum zonale which constitute here the most superior of the thalamic radiations going to the hinder part of the anterodorsal nucleus. They are more or less completely separated from nucleus lateralis A by part of the external medullary lamina. The possible relationships of this group of cells will be discussed when the subdivisions of the lateral nucleus have been described.

The nucleus lateralis A first appears in a transverse series between the anterodorsal and anteroventral nuclei (Pl. 1, fig. 6). It soon replaces the latter and comes to occupy the whole region lateral to the internal medullary lamina, capped dorsomedially by the nucleus anterodorsalis (the section illustrated in Pl. 1, fig. 7, is just posterior to the anterodorsal nucleus). Its cells are of medium size, fairly widely and evenly spaced. The boundary between it and the ventral nucleus is not very definite; in the latter the cells stain rather more deeply and are embedded in a denser network of myelinated fibres. The nucleus lateralis B comes to intervene between the nucleus lateralis A and the ventral nuclei, and can be distinguished by its smaller, more numerous and more irregularly disposed cells (Text-fig. 3).

The nucleus lateralis A extends posteriorly only a little beyond the anterior margin of the lateral geniculate body. Its posterior border is difficult to define but is clearer in a sagittal than in a transverse series (Text-fig. 3). On the deep surface of nucleus lateralis A, between it and the ventral nuclei, the smaller cells of nucleus lateralis B have already appeared. They now extend to the dorsal surface of the thalamus where they form the posterior boundary of nucleus lateralis A, a region characterized by the entry of several large blood vessels.

Nucleus lateralis B is very soon displaced from the surface by the appearance of the nucleus lateralis posterior at a level a little in front of the habenular commissure. It extends posteriorly for a considerable distance, covered dorsally by the nucleus lateralis posterior, separated laterally by the external medullary lamina from the lateral geniculate body (Pl. 1, fig. 8), and medially by the internal medullary lamina from the posterior part of the dorsomedial nucleus. Ventrally it rests on the ventral nucleus from which it cannot always be distinguished. When the dorsomedial nucleus disappears from the series it comes into contact with the parafascicular nucleus (Pl. 2, fig. 9), but by this level its boundaries are so indefinite that it cannot be separated from the

ventral nucleus with any confidence. Combined in this way with the ventral nucleus it can be followed posteriorly, lateral to the parafascicular and pretectal nuclei, and completely separated from the lateral geniculate body by the enlarged nucleus lateralis posterior. It is finally replaced by the nucleus suprageniculatus and the medial geniculate body.

The nucleus lateralis posterior (together with the lateral geniculate body) occupies about the posterior half of the dorsal surface of the thalamus (Text-figs. 1, 3, 4; Pl. 1, fig. 8; Pl. 2, figs. 9, 10). It extends forwards as a group of rather small cells further on the medial side than on the lateral, but the anterior boundary is not very precise. The extent of the dorsal surface is best marked by the fact that it is covered by fibres which appear to be passing from the optic tract to the tectum (Text-fig. 4). Bodian has shown that in the opossum by no means all these fibres are of retinal origin (1937). Below the surface it is penetrated by many similar fibres and also by fibres connecting the tectum with the lateral geniculate body or the cerebral cortex; these connexions cannot be determined with certainty in normal material.

The nucleus lateralis posterior shows two subdivisions, medial and lateral. The lateral subdivision, in preparations stained only for cells, cannot be distinguished from the medial part of the lateral geniculate body. It consists of medium-sized to small cells of the same type, but in sections stained by the Weigert method the dense network of fibres in the lateral geniculate body distinguishes it very clearly from the nucleus lateralis posterior. The medial subdivision consists of larger cells adjacent to the pretectal nucleus over most of its extent, but anteriorly related to the internal medullary lamina. The two subdivisions vary a little in their distinctness in different animals, but were recognized in all specimens examined; neither can be distinguished easily from the underlying nucleus lateralis B, except in Weigert preparations.

From the study of normal material no evidence was found that the fibre connexions of the nuclei of the lateral complex differ in any important respect from those described in other mammals. They clearly receive many fibres from the superior and intermediate thalamic radiations and are generally distinguishable from the ventral nuclei and the lateral geniculate body by the absence of any dense network of myelinated fibres between their cells. The relationship of the nucleus lateralis posterior to fibres of the optic system is very striking; its significance has been discussed by Bodian (1940) for the opossum, and his remarks probably apply to the phalanger.

If the small and variable group of cells which appears to correspond with Bodian's pars anterior is for the moment neglected, it is clear that the lateral nuclei of the phalanger correspond in all their main features with the description given for generalized placental mammals by Clark (1932), and in particular with the lateral nuclei of *Tupaia* (Clark, 1929*a*). They are as a whole perhaps more clearly differentiated than in *Tupaia*, and the subdivision of the nucleus lateralis posterior suggests comparison with the zones in that nucleus in *Tarsius* (Clark, 1930), although obviously there is no detailed correspondence.

In some placental mammals, e.g. the armadillo (Papez, 1932), the lateral complex is much less well developed than in the phalanger, but the same main parts are recognizable. The rat also does not seem to have advanced so far as the phalanger (Gurdjian, 1927); it may be noted that Gurdjian's nucleus ventralis pars dorsomedialis probably corresponds with the nucleus lateralis B of this description.

Following this same argument it would appear that Bodian's pars intermedia in the opossum corresponds with the nuclei laterales A and B of the phalanger, and that all parts of the lateral complex in the phalanger (with the exception of a possible representative of Bodian's pars anterior) are larger and more highly differentiated than in the opossum. This applies particularly to the nucleus lateralis posterior.

The significance of the cells which appear to correspond with Bodian's pars anterior in the opossum is doubtful. They were thought at first to belong to the lateral geniculate body; but they are in most cases separated from it by a short interval, they lack the characteristic network of fibres, and they fail to degenerate after cortical lesions which lead to almost complete degeneration of the lateral geniculate body. They may be a dorsal outlying group of cells belonging to the nucleus reticularis. They are smaller and less deeply stained than the cells typical of the nucleus reticularis, but have a very similar relationship to the thalamic radiations; they resemble, but at its dorsolateral extremity, the "nucleus reticularis medialis" of Bodian (1939). If these cells do indeed represent Bodian's pars anterior, and if the pars anterior corresponds, as he suggests (1939), with the nucleus lateralis A described in placental mammals, then the comparisons instituted above for the phalanger will need radical modification. Bodian's pars intermedia (my nucleus lateralis A and B) will presumably correspond with the nucleus lateralis B alone of *Tupaia* and other placental mammals, a very striking development for that nucleus in such primitive animals. Further evidence will be required to decide with confidence between these possibilities.

The ventral nuclei (Text-figs. 3, 4; Pl. 1, figs. 6–8; Pl. 2, fig. 9)

This complex extends throughout the whole ventral part of the thalamus from the posterior aspect of the anteroventral and anteromedial nuclei in front to the medial geniculate body and the nucleus suprageniculatus behind. Its separation from the lateral complex, never very clear, is practically non-existent in the posterior third of the thalamus.

Anteriorly the ventral complex is clearly separated from the anteroventral and anteromedial nuclei, but moulds itself into a depression between them (Pl. 1, fig. 6). This anterior boundary is accentuated by the presence of many fibres of the thalamic radiations. Dorsomedially the ventral complex is related to the internal medullary lamina and the paracentral nucleus, to the nucleus parafascicularis, and at its posterior end to the pretectal nucleus. The nucleus reuniens lies medial to the ventral complex in front, but in the posterior half of

the massa intermedia the ventral nuclei are continuous across the midline below the central nucleus. Behind this fusion the posterior paraventricular nucleus forms a medial and the nucleus subparafascicularis a ventral relation. Ventrolaterally it is related to the external medullary lamina, the nucleus reticularis and the zona incerta.

The following subdivisions can be distinguished:

(i) *Anterior* (Text-figs. 3, 4; Pl. 1, fig. 6). A well-defined band of small cells immediately behind the anteroventral and anteromedial nuclei, scattered among the very numerous fibres of the thalamic radiations in this region. It has rather the appearance of an intralaminar than of a principal thalamic nucleus, but unlike the intralaminar nuclei it degenerates completely after hemi-decortication.

(ii) *Intermediate* (Text-figs. 3, 4; Pl. 1, fig. 7). This part of the ventral complex lies ventromedial to the nucleus lateralis A and lateral to the hinder part of the nucleus reuniens. It is partially separated from nucleus lateralis A by nucleus lateralis B, and is interposed between the anterior and posterior parts of the ventral complex, not very clearly defined from the latter. It is characterized by the large size and deep staining of its cells, and also by the numerous bundles of thalamic radiation which it contains (Text-fig. 4). In some sections the most medial cells of this nucleus are slightly smaller and more closely packed than in other regions, but, in normal material at least, the difference is not sufficiently marked to justify the description of a separate medial part. The difference is not evident in Pl. 1, fig. 7.

(iii) *Posterior* (Text-figs. 3, 4; Pl. 1, fig. 8; Pl. 2, fig. 9). This is the largest subdivision of the ventral complex, and is continuous (as described above) with its fellow across the midline. In general its cells are smaller than those of the intermediate part. Its principal relationships can be seen in Text-fig. 3, and Pl. 1, fig. 8. Dorsolaterally, it blends with nucleus lateralis B, and posteriorly the two nuclei cannot be separated with any confidence, although the ventral nucleus generally contains more myelinated fibres.

The posterior part of the ventral nucleus can be further divided into dorsomedial and ventrolateral regions. The former, next to the internal medullary lamina, may represent a nucleus submedius. The latter is chiefly characterized by the numerous large bundles of fibres which it contains. From a level a little in front of the habenular commissure back to the nucleus parafascicularis the two regions are separated by a well-defined condensation of cells, smaller and rather more deeply stained than in other parts of the ventral nucleus (Text-fig. 3 and Pl. 1, fig. 8). This condensation forms a core in the posterior part of the ventral nucleus, and is the most definite of all the differentiations in this part of the thalamus. It contains a particularly dense network of fibres which appears to come, partly at least, from the lemniscus systems.

Behind the nucleus parafascicularis the posterior part of the ventral nucleus extends as a very ill-defined mass of cells, more or less completely blended with nucleus lateralis B, back to the suprageniculate nucleus and the

medial geniculate body (Pl. 2, fig. 9). These lie on its dorsolateral and lateral aspects respectively; the nucleus subparafascicularis is ventral to it. It is partly separated from the medial geniculate body by a layer of fibres, mainly constituted by the auditory radiations.

No important information concerning the connexions of the ventral nuclei could be obtained from this material. All parts have strong connexions with the intermediate and inferior thalamic radiations. The posterior part appears to receive the bulk of the fibres of the lemniscus systems, which enter it posteriorly, ventrally and laterally, but no analysis of these systems was possible. No lemniscus fibres were seen to enter the anterior part of the ventral nucleus. In spite of the broad fusion between the two ventral nuclei, commissural fibres are few in number.

There is obviously a fairly close correspondence between the ventral complex as a whole and the same region in the thalamus of other animals. There is no such close correspondence when the different subdivisions are considered. Before valid comparisons of a more or less detailed character can be made a more precise knowledge of the fibre connexions, and particularly of those from the lemniscus systems, is necessary. The following remarks must therefore be looked upon as provisional.

The pars anterior described by Bodian (1939) in the opossum seems to be the same as the anterior part in the phalanger. Its relationship to the anterior thalamic radiations is characteristic, and it is doubtful if a corresponding part has been described in the ventral nucleus of placental mammals. Bodian's pars medialis is represented in the phalanger by the medial region of the intermediate part, but is less clearly differentiated.

The intermediate and posterior parts in the phalanger correspond topographically with Bodian's pars principalis; this region of the thalamus appears therefore to show more intrinsic differentiation than in the opossum. The most striking feature of this differentiation is the appearance of a core of smaller, more closely packed, cells. This core resembles in some ways the pars arcuata of Carnivora (Rioch, 1929), but is less extensive; although in structure it is like the pars arcuata of primates (the posteromedial element in *Tarsius*, Clark, 1930) its situation is more anterior and it lacks the characteristic relation to the centre median nucleus. The latter is represented in the phalanger by the lateral part of the parafascicular nucleus. The difference between the pars arcuata of Carnivora and of primates was pointed out by Clark (1936). In general it seems that the ventral complex of the phalanger resembles that of the opossum more closely than that of other mammals, but that it has reached a higher level of differentiation which may in some respects parallel the differentiation of this complex in placental mammals.

Lateral geniculate body (Pl. 1, fig. 8; Pl. 2, fig. 9)

The lateral geniculate body is divided as usual into dorsal and ventral nuclei. It forms most of the lateral surface of the thalamus and is covered by

optic tract fibres. On its medial side the dorsal nucleus is related anteriorly for a short distance to the nucleus lateralis A, and through the rest of its extent to nucleus lateralis posterior and nucleus lateralis B. The latter is related only to the most ventral part of its medial surface and is more or less completely separated from it by the external medullary lamina, containing in this region the optic radiations. The medial limit of the lateral geniculate body (dorsal nucleus) is clearly marked throughout by the abrupt cessation of the dense network of fibres which it contains.

The dorsal nucleus of the lateral geniculate body is characteristically laminated, the laminae being superficial and concave medially. The concavity is filled with medium-sized cells, evenly distributed for the most part, and like those of the lateral part of the nucleus lateralis posterior. Four laminae can be recognized and doubtful traces of a fifth. The outermost (first) lamina is thin. It consists of rather large deeply staining cells, sparsely scattered beneath the optic tract fibres. Except for the doubtful fifth lamina, this is the least clearly defined of the laminae. The second lamina contains mainly smaller cells and a denser network of fibres than either the first or the third; there are a few large cells like those of the first lamina. The third is the thickest and most definite; its cells resemble those of the second, but tend to be orientated at right angles to the surface. Fibres are less numerous in this lamina. The fourth lamina does not differ appreciably from the second, and the fifth is represented only by the outer cells of the non-laminated core, which tend to be arranged parallel to the other laminae in some sections (e.g. Pl. 2, fig. 9). The laminae can be recognized throughout the whole anteroposterior extent of the dorsal nucleus of the lateral geniculate body; the fourth and fifth laminae do not extend into its ventral quarter, where the first, second and third laminae are present, but fuse indistinguishably with each other. In general the lamination varies considerably in its distinctness in different sections and in different specimens.

The ventral nucleus is co-extensive with the dorsal nucleus of the lateral geniculate body. Anteriorly and posteriorly it consists of small spindle-shaped cells; in the intermediate region the cells are larger. It is always clearly separated from the dorsal nucleus by a thin layer of fibres, and, although well developed, is much smaller than the dorsal nucleus. In a transverse section through about the middle of the lateral geniculate body, the ventral nucleus has an area between one-quarter and one-third of that of the dorsal nucleus; anteriorly and posteriorly it is proportionately smaller.

The ventral nucleus of the lateral geniculate body[1] is related anteriorly to the nucleus reticularis; posteriorly it extends medially above the cerebral peduncle into the zona incerta, where it has no clear boundary; it also extends beneath the anterior part of the medial geniculate body for a short distance (Pl. 2, fig. 10). Its surface is covered by optic tract fibres. Immediately beneath the surface is a region comparatively free from fibres, but the deeper or more

[1] Recent studies have made it very doubtful whether any of the optic tract fibres have a terminal connexion with the ventral nucleus of the lateral geniculate body.

medial parts contain a dense network which spreads into the zona incerta, and are also pierced by many bundles running vertically from the optic tract to the dorsal nucleus.

So far as can be judged from normal material, the connexions of the lateral geniculate body do not differ significantly from those of most mammals; no useful purpose would be served by attempting a detailed description till they have been analysed experimentally. The most striking feature of the dorsal nucleus of the lateral geniculate body of the phalanger is its lamination. In this it differs from most generalized mammals, both placental and marsupial. The lamination resembles that described in *Tupaia* (Clark, 1929*a*), but appears to be a little more distinct. In their regular medial concavity the laminae suggest comparison with the inverted type of lateral geniculate body in the Lemuroidea, e.g. in *Microcebus* (Clark, 1931).

Medial geniculate body (Text-figs. 1, 2; Pl. 2, fig. 10)

The medial geniculate body of the phalanger corresponds in all its features so closely with the opossum (Bodian, 1939) that it hardly needs separate description. Its superficial position can be seen in Text-fig. 1. It lies postero-lateral to the combined ventral nucleus and nucleus lateralis B, separated by a layer of fibres which probably consist partly of the auditory radiations, partly of corticotectal fibres. It is related dorsally to the nucleus lateralis posterior, and dorsomedially to the nucleus suprageniculatus which separates it from the pretectal nucleus.

It consists of a broad peripheral zone of medium-sized to small cells, well spaced, and best seen on the lateral and posterior surfaces. There is a central zone, not clearly defined from the peripheral, which extends anteromedially and consists of larger cells. These are more closely packed and vary somewhat in size, being smaller anteromedially than posterolaterally. The central zone contains many fibres; it receives the inferior brachium and gives rise to the auditory radiations.

As in the opossum, the ventral nucleus of the lateral geniculate body extends slightly beneath the medial geniculate body. Further caudally small fusiform cells are found in a similar position, which appear to be a lateral extension of the nucleus subparafascicularis as in the opossum (Bodian, 1939). Bodian has discussed the relationship of these cells to the ventral nucleus of the medial geniculate body described for other mammals; without a detailed comparative study it does not seem possible to come to a certain conclusion concerning their homologies in the phalanger.

Nucleus suprageniculatus (Pl. 2, fig. 10)

A small and rather poorly defined group of cells dorsomedial to the medial geniculate body, and posterior to the nucleus lateralis B. It is bounded dorso-laterally by the nucleus lateralis posterior, and dorsomedially by the pretectal nucleus from which it is separated by a layer of fibres. Its cells are of medium

size; they stain deeply and are arranged in irregular clumps. Although not a well-defined structure in the phalanger, it is clear that the nucleus suprageniculatus corresponds closely with the nucleus described by the same name by most authors; e.g. Bodian, opossum (1939), Clark, *Tarsius* (1930, 1933*b*), and Rioch, Carnivora (1929).

Pretectal nucleus (Text-figs. 1–4; Pl. 2, figs. 9, 10)

The pretectal nucleus cannot be classified in either of the main subdivisions of the thalamus. It consists of a broad band of cells between thalamus and midbrain, and is well developed in the phalanger; the cells are small, closely packed, and stain weakly with toluidine blue. It is very distinct in sections stained by the Weigert method, being filled with a dense network of myelinated fibres.

Anteromedially it lies behind the dorsal end of the fasciculus retroflexus, above the parafascicular nucleus, and medial to the large-celled part of the nucleus lateralis posterior (Pl. 2, fig. 9). As it extends posterolaterally it comes to lie lateral to the posterior commissure and the posterior paraventricular nucleus; the superior corpus quadrigeminum appears on its dorsomedial side. Ventrolaterally it is separated from the medial geniculate body by the posterior end of nucleus lateralis B and the nucleus suprageniculatus (Pl. 2, fig. 10). This ventrolateral surface is marked by a lamina of fibres. Ventrally, when the parafascicular nucleus disappears, it overlies the beginning of the tegmentum, and this boundary is not clearly marked. It ends finally between the medial geniculate body and the lateral border of the superior corpus quadrigeminum. The most caudal part can be differentiated from the rest of the nucleus; its cells are slightly smaller and it contains fewer myelinated fibres. This is the pars caudalis of Bodian (1939), and, as he says, it seems more closely related to the tectum than to the thalamus.

Superficially the pretectal nucleus is traversed by dense bundles of fibres many of which come from the optic tract (Text-fig. 4); some of these have been superficial throughout their course, others have pierced the lateral geniculate body and the nucleus lateralis posterior. Among them a number of large multipolar cells can be seen in some sections. These cells are not conspicuous in every specimen, but some representative of them can always be found. They constitute the large-celled nucleus of the optic tract. The deeper parts of the pretectal nucleus are traversed by fibres which come from the external medullary lamina, through the nucleus lateralis posterior. They are probably corticotectal fibres and they enter the deeper part of the superior corpus quadrigeminum (Tsai's layer 5 in the opossum, 1925). The pretectal nucleus is probably connected with the posterior commissure, and with the medial lemniscus (unless these are fibres of passage).

The pretectal nucleus as described here corresponds very closely with that of Bodian in the opossum and with the previous descriptions of Tsai (1925) and

Chu (1932*a*), but in the phalanger a representative of the large-celled nucleus of the optic tract has been included in it. This nucleus appears to be a much less definite structure than it is in *Tupaia* (Clark, 1929*a*), but in other respects the correspondence with this animal is again close.

No nucleus posterior thalami is described here for the phalanger. This term has been used in different ways by different authors. With some (e.g. Tsai, 1925; Chu, 1932*a*) the nucleus posterior seems to represent the hinder part of nucleus lateralis B (Bodian's pars intermedia), and in Carnivora (Rioch, 1929) it is more closely related to the lateral than to any other group of nuclei. In the rat (Gurdjian, 1927) and the armadillo (Papez, 1932) it probably represents the deepest part of the pretectal nucleus described here, as it does in the macaque (Aronson & Papez, 1934). One may conclude provisionally that the nucleus posterior thalami is the deeper part of the pretectal nucleus of primitive mammals. Whether its degree of differentiation in more advanced mammals is enough to justify its description as a separate nucleus cannot be decided here.

DISCUSSION

The most obvious conclusion which can be drawn from this description of the thalamus in the phalanger is that it resembles closely the thalamus of primitive placental mammals.

The midline and intralaminar nuclei are built on essentially the same plan as in the placental mammals. Most of the differences which have been noted are in matters of detail where the personal factor before mentioned (p. 198) must have considerable importance. For example, the subdivision of the nucleus reuniens into a nucleus subparataenialis and a nucleus reuniens anterior and posterior, the presence or absence of a nucleus submedius, the separation of the rhomboid nucleus from the mass of cells that has been described here under the one name, nucleus centralis, are all differences of this type. In the absence of close collaboration between workers, with interchange of actual material, such comparative descriptions must be to some extent arbitrary. Except on a few points it is possible to conclude only that the nuclei of this group are well developed in the phalanger, as one would expect in a primitive mammal, but perhaps a little less highly differentiated than in the opossum. They unquestionably form a larger and more conspicuous feature in the thalamus than in the higher primates or in the Carnivora. Their general lack of clear differentiation from adjacent principal nuclei is in favour of Papez's suggestion (1932) that they are a residuum of the matrix from which the principal nuclei have been developed, a suggestion which is particularly applicable to the dorsomedial nucleus. Another point which may be stressed is the poor development of commissural fibres in the massa intermedia. Technical failure in staining or impregnation might account for this observation, but there seemed no reason why this should be likely. Apart from such a possibility, there is no doubt that commissural fibres are very much better developed in the

opossum (Bodian, 1940) and probably in most primitive placental mammals. A lack of commissural fibres is more characteristic of higher mammals.

Concerning the dorsomedial subdivision of the thalamus, little comment is necessary beyond what has already been given in the descriptions of individual nuclei. It may be added that the comparatively large size of the parataenial nucleus is characteristic of primitive mammals; more important is the appearance of a small-celled differentiation in the dorsomedial nucleus comparable to the main part of the dorsomedial nucleus which in primates projects on to the prefrontal cortex.

The anterior nuclei present no striking peculiarities. In all their important features they resemble those of other mammals, and there is no outstanding development of one or other of the three nuclei. The relationship of the anterodorsal nucleus (and particularly its commissural part) to the internal medullary lamina and its nuclei suggests the possibility that it should be classed with the intralaminar nuclei. This is not by itself an adequate reason for modifying the usual classification, which is based on a common relationship to the mammillothalamic tract. It is true that in this material no fibres could be traced from the mammillo-thalamic tract to the anterodorsal nucleus, as Bodian found also in the opossum (1940); there is however evidence that such connexions exist in some placental mammals at least (Clark, 1932). Experimental methods have also demonstrated that, unlike the intralaminar nuclei, it has cortical connexions (e.g. in the cat, Clark, 1933*a*; Waller, 1938), although they may be less abundant than those of the other two anterior nuclei. Unless, therefore, further work can show some more fundamental difference between the anterodorsal nucleus on the one hand and the anteroventral and anteromedial nucleus on the other, the three must remain classified in one group. In the proportionate size of the anterior group of nuclei as a whole, and in its relationship, particularly to the ventral nuclei, the thalamus of the phalanger corresponds closely with that of primitive placental mammals.

So far the differences noted between the thalamus of the phalanger and of the opossum and other mammals have been for the most part matters of detail and of no clear significance; a few, e.g. the lack of commissural fibres, and the differentiation of the dorsomedial nucleus, have suggested a more advanced stage of development. This suggestion is confirmed by conditions found in the remaining parts of the thalamus.

The nucleus lateralis posterior, for example, is far better developed than in the opossum, the rat (Gurdjian, 1927) or the armadillo (Papez, 1932). It seems to be on the way towards the formation of a pulvinar visible to external examination, and is therefore a progressive feature. Its differentiation into medial and lateral parts is also suggestive of the more elaborate subdivisions of the pulvinar which make their appearance in higher mammals, but it would be unwise to put much reliance on this feature till it has been studied by experimental methods.

The appearance in the ventral complex of nuclei of a region which may be

comparable with the pars arcuata in other mammals again suggests parallel development of a progressive kind, although in other respects there does not appear to be much resemblance between the subdivisions of the ventral complex and those in placental mammals. Obviously a more precise knowledge of fibre connexions is essential in this region.

The most definite evidence of all is to be found in the lateral geniculate body, where the laminated arrangement of the cells indicates a degree of differentiation in the visual system which is considerably higher than in any of the described primitive mammals except *Tupaia*. It seems in fact to have reached a slightly higher level than in *Tupaia*, approaching the lemuroid condition. This elaboration of the visual system is also reflected in the large relative size of the nucleus lateralis posterior and the superior corpus quadrigeminum. The pretectal region is, however, typically primitive, and it is doubtful if the appearance of a rudimentary large-celled nucleus of the optic tract has much significance.

Taking these facts together there seems to be considerable justification for saying that the thalamus of the phalanger shows marked progressive tendencies, clearly parallel to tendencies that have been more fully realized in placental mammals. The parallelism is perhaps closer to primitive members of the Order Primates than to any other group, as one might expect on functional grounds. Most of the primitive primates are nocturnal animals, with a well developed visual sense, and arboreal in habit; the phalanger is like them in all these respects.

There is in the thalamus of the phalanger a notable absence of any features which might be described as typically marsupial, and which would differentiate it from all placental mammals. There are two possible exceptions to this statement. The relationships of the nucleus lateralis pars anterior of Bodian have been discussed, and while admitting that the evidence at present available is inadequate for a firm conclusion, it seems possible that this nucleus may be a differentiation peculiar to marsupials. The same may apply to the nucleus ventralis, pars anterior, which, in its intimate relation to the anterior nuclei and to the thalamic radiations in this region, does not seem comparable to the anterior part of the ventral nucleus in placental mammals. Neither of these features can be considered very important without the confirmation of more detailed evidence. It should perhaps be added that an extensive study in a large number of marsupials might well reveal common features, not shared by placental mammals, which, seen only in one marsupial, could not be considered significant. It is likely, however, that such common features, were they observed, would be matters of detail, and would not invalidate the conclusions drawn from this and from Bodian's description: that the marsupial thalamus is essentially similar to the placental thalamus, both in fundamental structure and in the evolutionary tendencies shown by more advanced marsupials when compared with the more primitive.

SUMMARY

The thalamus of *Trichosurus vulpecula* has been described on the basis of serial sections of normal brains stained by toluidine blue, a modification of the Weigert method, and by Davenport's silver impregnation method.

In structure it is essentially similar to the thalamus of *Didelphys virginiana* (Bodian, 1939, 1940), and to that of primitive placental mammals; it shows however a number of progressive features in which it resembles rather closely the published account of *Tupaia* (Clark, 1929*a*).

These progressive features are shown most clearly in the following observations. The lateral geniculate body (dorsal nucleus) is laminated as in higher mammals. The nucleus lateralis posterior (the equivalent of the pulvinar) is large, and differentiated into medial and lateral parts. A possible representative for the "pars arcuata" has appeared in the ventral complex. There is a well-marked lateral differentiation in the dorsomedial nucleus which appears to correspond with the main part of that nucleus in the higher primates. Commissural fibres between the two sides are feebly developed. In nearly all other respects the structure is that typical of primitive mammals in general, and no undoubted marsupial specializations were observed.

The work is intended to serve as a basis for future experimental investigation.

ABBREVIATIONS USED IN FIGURES

AD	Nucleus anterodorsalis.
AM	Nucleus anteromedialis.
AV	Nucleus anteroventralis.
C	Nucleus centralis.
CL	Nucleus centralis lateralis.
DM	Nucleus dorsomedialis.
EML	External medullary lamina.
HCo	Habenular commissure.
LA	Nucleus lateralis A.
LB	Nucleus lateralis B.
LGBd	Dorsal nucleus of lateral geniculate body.
LGBv	Ventral nucleus of lateral geniculate body.
LM	Lemniscus medialis.
LP	Nucleus lateralis posterior.
LPL	Nucleus lateralis posterior, lateral part.
LPM	Nucleus lateralis posterior, medial part.
MGB	Medial geniculate body.
OTr	Optic tract.
PC	Nucleus paracentralis.
PCo	Posterior commissure.
PF	Nucleus parafascicularis.
Pr	Nucleus pretectalis.
PT	Nucleus parataenialis.
PVA	Nucleus paraventricularis anterior.
PVP	Nucleus paraventricularis posterior.
R	Nucleus reuniens.
Rt	Nucleus reticularis.
SCQ	Superior corpus quadrigeminum.
SG	Nucleus suprageniculatus.
SPF	Nucleus subparafascicularis.
STh	Nucleus subthalamicus.
VA	Nucleus ventralis, anterior part.
VI	Nucleus ventralis, intermediate part.
VP	Nucleus ventralis, posterior part.

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EXPLANATION OF PLATES 1 AND 2

The illustrations (figs. 5–10) in Pls. 1 and 2 are all untouched photographs of 40 μ transverse sections through the thalamus of *Trichosurus vulpecula*. They were all cut in celloidin and stained with toluidine blue. The magnification is $\times 12$. The approximate levels of the different sections are shown in Text-figs. 1 and 2.

Plate 1

Fig. 5. A section through the posterior part of the anterior group of nuclei.

Fig. 6. A section showing the most anterior parts of the lateral, ventral, and dorsomedial nuclei. It is 16 sections posterior to fig. 5.

Fig. 7. A typical section through about the middle of the dorsomedial nucleus. The intermediate part of the ventral complex is particularly well shown. It is 14 sections posterior to fig. 6.

Fig. 8. A section at the level of the habenular commissure. This section shows the condensation of cells (? pars arcuata) in the posterior part of the ventral complex.

Plate 2

Fig. 9. A section at the anterior end of the posterior commissure. It shows the structure of the lateral geniculate body particularly well.

Fig. 10. A section through the extreme hinder end of the thalamus, passing through the medial geniculate body; the nucleus subparafascicularis is scarcely visible on account of the pale staining characteristic of its cells.

Fig. 6.

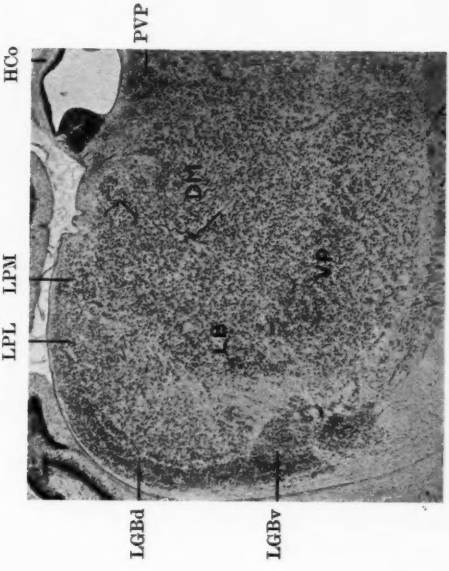

Fig. 8.

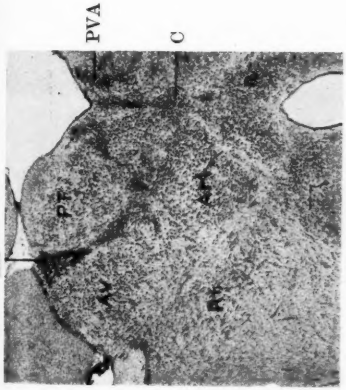

Fig. 5.

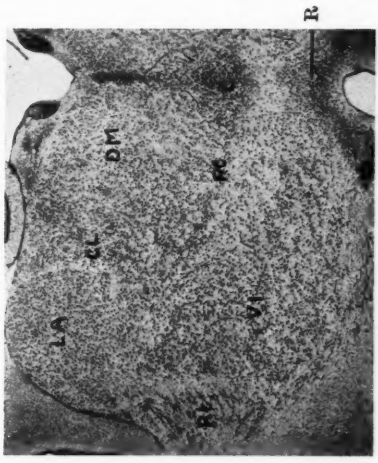

Fig 7.

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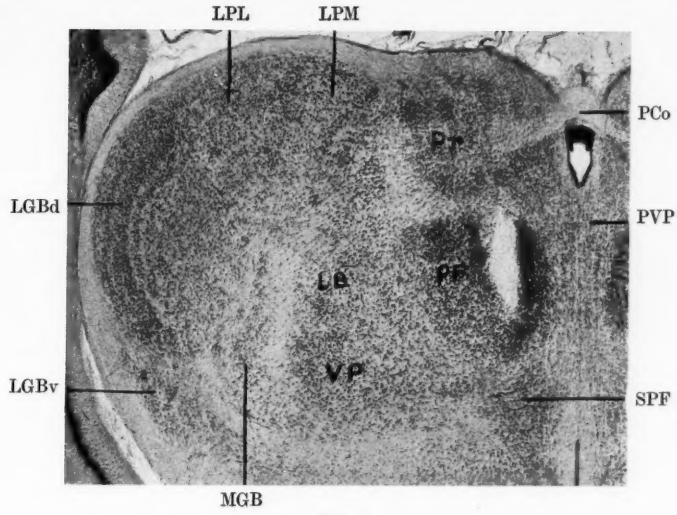

Fig. 9.

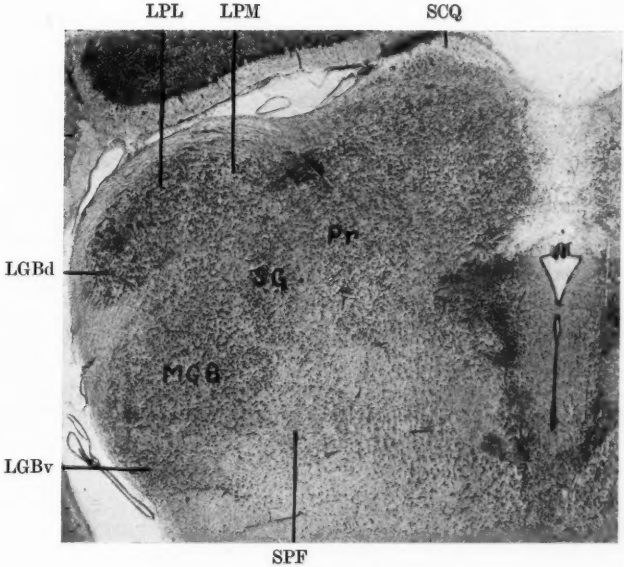

Fig. 10.

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OBSERVATIONS ON THE ASSOCIATION FIBRE SYSTEM OF THE VISUAL CORTEX AND THE CENTRAL REPRESENTATION OF THE RETINA

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It is generally assumed, on the basis of experimental studies (as well as traditional teaching), that the visual cortex is directly connected with distant cortical areas, particularly with those of the parietal lobe, by well-marked systems of association fibres. It has been assumed, also, that these connexions provide the means whereby visual stimuli can immediately be brought into relation with the general sensory or the auditory cortex so as to provide the basis of visuo-tactile or visuo-auditory associations. Some authors have even described direct fibre paths extending from the visual cortex to the motor cortex of the precentral convolution.

Some doubt regarding these supposed connexions was aroused several years ago as the result of an experiment in which almost the entire parietal cortex was excised in a monkey (Clark & Northfield, 1937). This animal was allowed to survive for 2 months, by which time it was anticipated that definite cytological changes would be apparent in the visual cortex of the operated side, consequent on the interruption of all the association fibres which are presumed to pass from it into the parietal areas. It was a matter of some surprise to find, however, that the cytoarchitecture of the visual cortex showed no demonstrable change at all. The lamina pyramidalis in particular, to which associational functions are frequently assigned by neuro-anatomists, showed the normal density of cells. This observation suggested the need for a re-examination of the association fibre system of the visual cortex with the aid of the Marchi technique, and, in order to eliminate all possible errors of interpretation, it was planned in the experiments to produce only small and quite circumscribed lesions, with a minimal involvement of subcortical tissues. If facilities and experimental material had permitted it, the intention was to accumulate a large number of such experiments, covering as much as possible of the striate area of the brain. In fact, three experiments only were completed; nevertheless, the results of these proved to be sufficiently definite to allow of record and discussion.

METHODS

Those who have used the Marchi method for demonstrating degenerating nerve fibres are well aware of the difficulties and fallacies of the technique. Yet it is still desirable to emphasize the great care which is needed in its application to the results of intracranial operative lesions. However carefully such an operation is carried out, parts of the brain besides that actually involved in the

experimental lesion must be subjected to some degree of trauma. This may occur during the actual exposure and manipulation of the brain or during the post-operative healing process. Thus the pressure of the margins of the bone flap against a congested or an even slightly oedematous brain frequently leads to local injury, and the contraction of adhesions may further involve the surface of the cortex some distance from the site of the lesion; or the animal in the recovery stage may knock the operated part of the head against the side of the cage, producing a local bruising of the underlying brain. Lastly, even a mild inflammatory reaction can quite easily lead to disturbances of the more superficial neurones sufficient to give rise to some undesirable Marchi degeneration. Gross lesions of the brain are always unsuitable for the Marchi method because it is difficult, and indeed usually impossible, to determine how far pathological disturbance may have extended beyond the lesion as the result of unintentional trauma and inflammatory reaction. It seems certain that such disturbing factors have frequently led investigators to describe direct fibre connexions which do not really exist. Consequently, for Marchi preparations on the brain itself it is essential (1) that the operation should be carried out with the utmost delicacy and all the stringency of aseptic technique, and (2) that only small, single and perfectly circumscribed lesions should be produced.

The application of the Marchi technique also demands the closest attention. The brain should be fixed *in situ* by the intravascular injection of 10% formalin, and its removal from the skull requires to be undertaken with great care. The formalin must be thoroughly washed out of the blocks of tissue before treatment with osmic acid, and at every stage in the staining and embedding the material should be manipulated with extreme delicacy. In spite of every precaution it will sometimes happen that the sections show a pseudo-Marchi reaction which may be widespread or localized in patches. Hence the sections themselves require to be interpreted with a critical eye. Evidence of injury or pathological change in the brain apart from the actual lesion must be carefully looked for as a possible source of the Marchi reaction, and in dealing with individual fibres it is important to make certain that the osmic staining in or along a blood vessel is not mistaken for a degenerating myelin sheath. Lastly, it is always essential to have available for study a complete series of serial sections so that degenerating fibres can be followed *in continuity* from their origin to their destination. If this precaution is neglected, local deposits of osmic granules related to unintentional trauma, or the result of a pseudo-Marchi reaction, may be taken as an indication of the course of fibres whose origin has been interrupted by the experimental lesion.

These experiments were carried out in rhesus monkeys. The occipital lobe was exposed by turning down a bone flap under nembutal anaesthesia. A lesion was in each case produced on the exposed surface of the visual cortex by devascularization of a small area (about 5 mm. in diameter) of the cortex. This method of devascularization results in the destruction of the cortex and the

immediately subjacent white matter only, and thus minimizes possible damage to deeper structures. The animals were kept alive for 14–15 days and then killed by washing through the circulation with normal saline under ether anaesthesia. The saline was immediately followed by the injection of 10% formalin.

In each case the posterior half of the corresponding cerebral hemisphere was sliced into four horizontal blocks and from each of these a continuous series of serial sections was prepared.

RESULTS

Monkey 60*a*. Operated 9 May 1939, killed 24 May 1939.

A small lesion was made close to the lunate sulcus. Measured in the sections the lesion is found to measure 3 mm. in sagittal diameter, its anterior margin being 2·5 mm. from the lip of the occipital operculum. It extends down to the white matter, involving only the immediately subcortical layer.

The main fasciculus of degenerating fibres which arise at the site of the lesion can be seen to turn caudally to the attached base of the operculum and then to curve medially to reach the tapetal layer of white matter. At this point some of the fibres turn forwards in the tapetum and are presumably callosal fibres. Others pass through the tapetum, cutting across the main current of fibres in this layer, and reach the internal sagittal stratum of the occipital lobe. They then run forwards, close alongside the posterior horn of the lateral ventricle.

Apart from this main fasciculus there is a finer, diffuse Marchi deposit in the subcortical white matter extending in front of and behind the lesion. In front it reaches the lip of the occipital operculum, and in this part of the visual cortex degeneration is shown in some of the radial or vertical fibres. With the possible exception, however, of its margin where it adjoins the visual cortex, the peristriate cortex (area 18) is free of Marchi deposit. Caudal to the lesion, radial fibres stained with fine osmic granules can be seen in the cortex, but these extend back only for about 3 mm. Immediately behind the lesion they penetrate to the pyramidal layer; further back they can only be traced into the deepest cortical layer. In the subcortical white matter the Marchi deposit reaches still further—to a distance of 13 mm. behind the lesion. It is doubtful, however, whether any of this is related to the primary lesion, for in some sections it is apparent that a superficial abrasion on the surface of the occipital cortex has been caused by the pressure of the bone flaps at a level 15 mm. posterior to the occipital operculum. Here the cortex bulges up slightly and degenerating fibres can be seen extending from the summit of the bulge to the subcortical white matter.

The calcarine cortex on the medial surface of the occipital lobe is entirely free of Marchi staining. At the summits of the gyri which in horizontal sections are seen to lie between the lunate sulcus, the lateral sulcus, the intraparietal sulcus and the central sulcus, an occasional degenerating fibre is present, and in

some parts the subcortical white matter shows a diffuse stippling of osmic granules. There is no doubt at all that this reaction is the result of some slight trauma to the exposed surface of the cerebral hemisphere during the operation or during the post-operative healing. For there is no degeneration in the white matter deep to the intervening sulci, and if the serial sections are followed there is seen to be no continuity between the Marchi degeneration extending from the lesion and the occasional deposit seen in the parietal cortex. Certainly none of the degenerating fibres in the tapetum turn laterally into the peristriate cortex.

Monkey 61*a*. Operated 13 May 1939, killed 27 May 1939.

In this case the lesion was placed in the middle of the occipital cortex. It measures 5 mm. in sagittal diameter and is situated 11 mm. behind the opercular lip. The sections show a local bulging at the site of injury which happened to be close to the margin of the bone flap.

The sections show that the lesion has been complicated by a local inflammatory process which has extended into the subcortical white matter and led to a local disorganization so that the Marchi degeneration is certainly more extensive than would have resulted from the primary lesion alone. Moreover, there is a small inflammatory exudate in the posterior horn of the lateral ventricle, and some congestion in the pial tissue on the surface of the occipital cortex. There is a dense deposit of very coarse granules in the white matter deep to the lesion. In front, for a distance of 4 mm. or so from the anterior margin of the lesion, degenerating radial fibres can be seen in the visual cortex extending into the internal granular layer and the infragranular layers. There is no Marchi degeneration in the free lip of the occipital operculum. Behind, degenerating radial fibres in the cortex extend back from the lesion for about 3 mm. Coarse degenerating fibres can be traced further back in the subcortical white matter, but they cannot be followed into the cortex itself. Possibly they terminate in the deepest layer and represent callosal fibres which have been involved by the lesion. The latter has certainly affected the white matter related to a submerged part of the visual cortex on the lateral aspect of the occipital lobe, and here, also, degenerating fibres are present in the cortex.

Apart from the regions of the visual cortex already mentioned, Marchi degeneration is only found in one other part of the cortex, that is, in that part of the peristriate area which is submerged and overlaid by the free lip of the occipital operculum. Here the osmic deposit is rather conspicuous in the subcortical white matter, and individual degenerating fibres can be traced into the substance of the cortex over a width of about 2 mm. The gyri of the parietal lobe which lie between the lunate sulcus and the central sulcus are entirely free of Marchi deposit.

Monkey 63*a*. Operated 16 May 1939, killed 30 May 1939.

The lesion is extremely small in this animal, measuring 1·5 mm. in sagittal diameter and situated 1·5 mm. behind the extreme tip of the occipital oper-

culum. It is almost entirely confined to the cortex, but a minute cavity filled with exudate extends into the subjacent white matter, evidently involving here some of the afferent fibres passing forwards to the opercular lip.

Coarse degenerating fibres extend back from the lesion to the attached base of the occipital operculum, and here most of them appear to curve medially to enter the tapetal layer of white matter. They then run forwards, but none of them turn superficially again into adjacent areas of cortex. Indeed, the gyri in front of the lunate sulcus are entirely free of any osmic deposit. A sprinkling of Marchi granules can be traced back from the lesion in the subcortical white matter of the occipital lobe for a distance of about 15 mm., and for a distance of 4–5 mm. behind the lesion scattered degeneration is to be seen in the radial fibres of the cortex itself. Further caudally no Marchi degeneration is present in the substance of the visual cortex. In front of the lesion some degenerating radial fibres are also to be seen in the cortex of the opercular lip.

DISCUSSION

In all these three experiments in which the lesions in the visual cortex were deliberately made small and circumscribed in order to avoid complications, the resulting Marchi degeneration was very pronounced in efferent cortical fibres. From their position these appear to belong to two systems—those passing into the corpus callosum and which entered the tapetal layer of white matter, and those which were presumably destined for the brain-stem, i.e. cortico-tectal, cortico-pontine, and possibly cortico-geniculate fibres. It is of some importance to note that the Marchi reaction was quite strong in these fibres, extending from the site of the lesion into the centrum ovale as far as the limits of the sections (Fig. 1). There can thus hardly be any question that other fibres involved by the lesion were not apparent because the osmic staining was not sufficiently intense.

In one experiment (63*a*) the whole of the cortex in front of the lunate sulcus was entirely clear of any osmic deposit. In other words, it appears that no efferent association fibres are given off from the visual cortex at the site of the lesion to any part of the parietal lobe. In experiment 60*a*, a similar lesion also produced negative results as regards such association fibres. In this case, however, a rather conspicuous reaction was observed in a short strip of submerged peristriate cortex lying under cover of the lip of the occipital operculum. It seems probable therefore that association fibres from the site of this particular lesion may project on to this cortical area. But if this is so, the projection of the association neurones appears to be very limited. Certainly in none of these experiments is there any suggestion of a massive system of association fibres extending from the visual cortex to adjacent areas of the parietal lobe, and even to the motor cortex as some authors have stated to be the case. Indeed, we are led to suspect that such long association paths from the visual cortex are completely absent in the monkey and that those which have been described have their origin in inadequately controlled experimental material.

In all these experiments the Marchi reaction showed evidence of quite short association fibres running from the site of the lesion into the immediately adjacent part of the visual cortex. These fibres are components of the radial fasciculi of the cortex which can be traced from the white matter into the cortical substance, and in the Marchi material they were marked along their course with fine osmic granules. In none of the experiments, however, did such association fibres extend for a distance of more than 4 or 5 mm. from the lesion. It has previously been suggested that the radial fibres of the cortex are predominantly associational in function (Poliak, 1932), and this interpretation

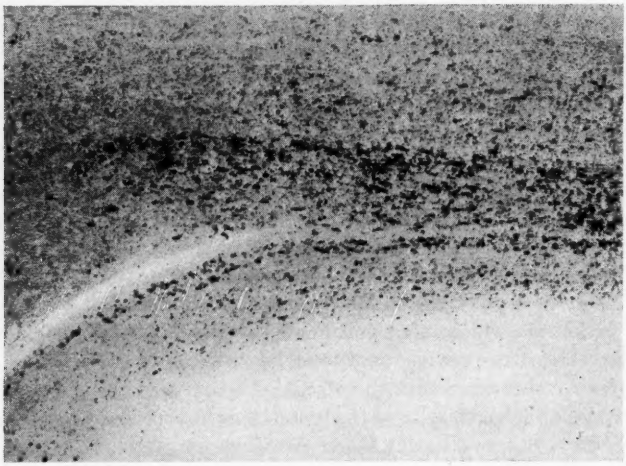

Fig. 1. Photomicrograph of a section from experiment 61*a*, showing the Marchi reaction in the corticifugal fibres which have been involved by a small and localized lesion of the visual cortex. × 58. The position of the lesion (not shown in the picture) is immediately to the right. Above, fine degenerating fibres are seen running through the deeper layers of the visual cortex adjacent to the lesion. Below, the visual cortex which covers the deep aspect of the occipital operculum is seen to be quite free of Marchi degeneration.

receives support from the study of structural changes in isolated portions of the visual cortex (Clark & Sunderland, 1939). In the latter work, however, it was found that only a small proportion (about 15 %) of the fibres in the radial fasciculi of the visual cortex underwent atrophy when a narrow strip of cortex 6 mm. in width had been isolated for over 4 months. It appears therefore that the majority of the fibres in each radial fasciculi must have their origin in cells which lie within the cortex not more than a few millimetres distant. In other words, these observations are consonant with the results of the experiments recorded in the present paper, that direct association fibres from any spot on the visual cortex in the monkey extend for extremely short distances. Certainly there is no evidence that they extend over all or almost all parts of the visual cortex as has been claimed by some investigators.

It is interesting to compare these conclusions with the results of recent electro-physiological studies of the cerebral cortex. Combining the method of local strychninization with the recording of electrocorticograms, the late Dr Dusser de Barenne and W. S. McCulloch have worked out the interconnexions of the sensory and other cortical areas in the monkey. In some areas local strychninization produces characteristic "spikes" not only in the electrocorticogram of the minute area strychninized, but also in other cortical areas. For example, local strychninization of area 7 produces distant effects in areas 5, 2, 1 and 4, and the authors adduce evidence proving that these effects are produced by impulses which travel along axones in the subcortical white matter (though this does not preclude the possibility that entirely intracortical neurones may also play a part in the diffusion of impulses, at least to a limited distance). In sharp contrast to the general sensory cortex it was found that in the visual cortex (area 17) the "strychnine spikes" are entirely restricted to the area strychninized and its immediate vicinity, extending for a distance of not more than 1 or 2 mm. These results appear to indicate that direct association neurones arising at any one point in the visual cortex extend only for a few millimetres before they terminate again in the immediately adjacent cortex; for if there were any considerable association tracts leading to more distant parts of the visual cortex or to other cortical areas of the parietal lobe, then these tracts ought to conduct the strychnine excitation and the latter should be detected in these areas. Thus the results of electrophysiological studies are also in agreement with the Marchi experiments recorded in the present communication.

It now appears that the effect of any retinal stimulus which reaches the visual cortex cannot be communicated by direct fibre connexions to all parts of the visual cortex, or to distant areas of the parietal lobe. On the contrary, the immediate effects of the stimulus must be very localized spatially. In the light of these observations, it is of some interest to consider certain aspects of the cortical projection of the retina in relation to cortical organization.

It is well known that the lateral geniculate body is the relay station for projecting retinal impulses on to the visual cortex and that in all the higher primates it consists in a great part of its extent of six well-defined layers of cells (see the diagram in Fig. 2). Many years ago Minkowski showed (by the study of trans-neuronal cell-atrophy) that layers 1, 4 and 6 receive fibres from the opposite eye, and layers 2, 3 and 5 from the eye of the same side. Later work (Clark & Penman, 1934) confirmed and amplified this observation; further (although the importance of this point was not recognized at the time) it also showed that the smallest lesion in the retina which produced any detectable cell changes in the lateral geniculate body always involved a group of cells (of approximately equivalent size) in all of the corresponding three layers, these cells being disposed as a straight band radiating from the hilum of the lateral geniculate body to its convex deep surface. In other words, it seems that the *receptive unit* of the lateral geniculate body in respect of each retina is a

narrow band of cells radiating from the hilum, and involving either the cell laminae 1, 4 and 6 (heterolateral optic fibres) or 2, 3 and 5 (homolateral optic

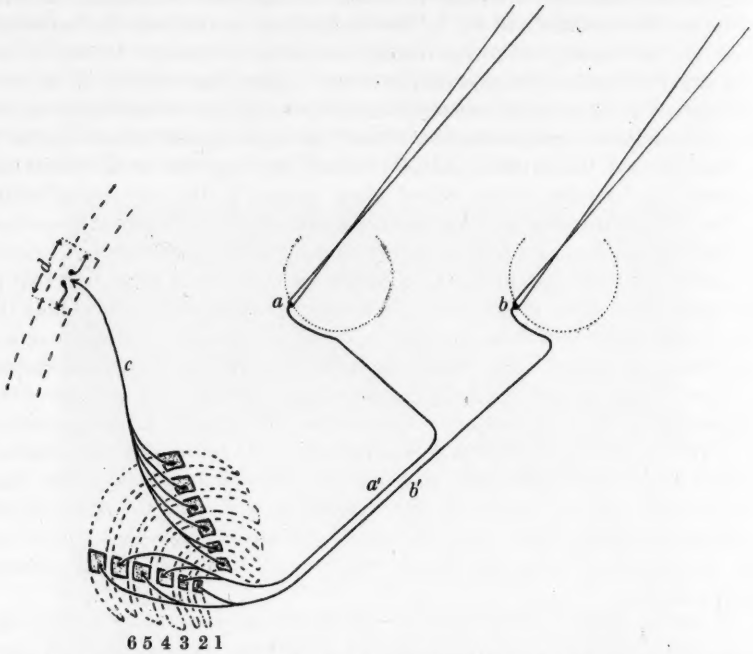

Fig. 2. Diagram illustrating certain points in the central representation of the retina. Fibres from equivalent spots (*a*, *b*) in the two retinae pass back in the optic tract to the same region of the lateral geniculate body. Crossed fibres (*b'*) terminate in laminae 1, 4 and 6 and uncrossed fibres (*a'*) in laminae 2, 3 and 5. Thus the *receptive unit* in the lateral geniculate body with respect to each retina is a band of cells radiating from the hilum of the nucleus, and involving three laminae. On the other hand, the *projection unit* of the lateral geniculate body is a band of cells involving all six laminae. From these cells fibres run together (*c*) to reach the same spot on the visual cortex. Thus the lateral geniculate body may be regarded as a mechanism which makes it possible for impulses from corresponding points in the field of vision of each eye to be brought into relation with each other at the point where they impinge simultaneously upon the cortex. It is also speculatively suggested in the text that, if the three laminae related to each retina are concerned with the three fundamental colour sensations postulated by the trichromatic theory of Young and Helmholtz, the lateral geniculate body in the monkey (as also in man) provides a mechanism for the fusion of impulses (at each single point in the cortex) which is necessary for the recognition of colour mixtures.

On arrival at the cortex the retinal impulses can be immediately diffused by association fibres, but it appears that the latter only extend over a relatively small area (*d*) with a diameter of probably not more than 10 mm., and perhaps considerably less.

fibres). From this observation it may be inferred *either* (1) that each single retinal fibre on arrival at the lateral geniculate body divides into three terminals, each passing to one of the three corresponding cell laminae, *or* (2) that the

conducting unit in each optic nerve in respect of the geniculate body consists of three fibres arising from adjacent ganglion cells of the retina, and that of these three fibres (in the case, for example, of crossed fibres) one terminates in cell lamina 1, a second in lamina 4, and the third in lamina 6. This problem can only be settled ultimately by direct neurohistological studies, though it is possible that a numerical computation of the ratio between the number of crossed and uncrossed fibres in each optic tract and the number of cells in each set of three laminae in the lateral geniculate body might provide indirect evidence. Such neurohistological studies of the lateral geniculate body as have been made (and also the author's own studies of silver impregnated material) provide no evidence for the first alternative, and it seems almost certain that the second is the more correct. The possible significance of the latter in relation to the Young-Helmholtz theory of colour vision has been noted elsewhere (Clark, 1940). This theory invites the assumption that there are three different types of sensory element in the retina, the excitation of which gives rise respectively to the sensations of red, green and violet. If this assumption is true, then the conducting unit of the optic nerve (so far as colour vision is concerned) must also consist of three fibres taking origin from adjacent neurones in the retina. In other words, our experimental studies of the lateral geniculate body in monkeys have demonstrated a possible anatomical basis for the Young-Helmholtz theory. It is perhaps worth noting that in the monkey (and apparently in man also) the six layers of the lateral geniculate body are only fully differentiated in that part of the nucleus which receives impressions from the central part of the visual field. In those parts of the nucleus which are related to extreme peripheral vision, in which the recognition of colour distinction is difficult or impossible, the six laminae of the lateral geniculate body become merged into each other to a considerable extent. Lastly, it may be mentioned that a well-defined six-layer formation is only found in the lateral geniculate body of primates, perhaps in correlation with the fact that this is the only group of mammals which has been shown by properly controlled tests to possess full colour vision.

If corresponding points on the two retinae are simultaneously stimulated, then activation of cells in the geniculate body will occur along a radiating band which involves all six of the cell-laminae in approximately equal proportions. This is also the *projection unit* of the lateral geniculate body in relation to the cortex, for Poliak (1933) has shown that the smallest lesion (not much more than 1 sq. mm.) in the monkey's visual cortex which leads to detectable changes in the geniculate body results in such a narrow radiating band of cell-atrophy involving in each section a small cluster of cells in each of the six laminae.

In spite of arguments put forward by Henschen (1925), it is not known whether any mechanism exists in the lateral geniculate body which would provide the anatomical basis of a fusion of crossed and uncrossed retinal impulses. Henschen notes that a small lesion in the geniculate body produces

bilateral scotomata, but it would hardly be possible for such a lesion to be limited to a single cell lamina without even involving optic fibres passing to adjacent laminae. Henschen describes (in the human brain) a type of nerve cell in the geniculate body whose processes dominate a territory more extensive than the distances between two or three cell laminae, and he supposes that these cells are able to establish connexions between receptive cells related to corresponding retinal areas of both eyes. However, the presentation of his neurohistological evidence is not entirely convincing and requires confirmation. Certainly, as Poliak has pointed out, the fact that complete ablation of the visual cortex in the monkey leads to a total atrophy of *all* the cells of the lateral geniculate body seems to decide finally against the existence in the nucleus of any intercalated neuronal elements whose function might be presumed to link up adjacent cell laminae. Lastly, the sharp contrast shown between the three laminae affected by transneuronal atrophy and the three normal laminae after section of one optic nerve suggests a very considerable degree of functional independence in the two sets.[1]

If the impulses from the two retinae remain distinct within the limits of the lateral geniculate body, they must be brought into the most intimate relation as soon as they are projected on to the cortex, for, as already noted, each spot of the visual cortex receives impulses from corresponding sections of all six cell laminae. It has been suggested that the two layers of cells which comprise the lamina granularis interna of the visual cortex are concerned with receiving crossed and uncrossed retinal impulses respectively. There is, however, no evidence for this conception. A recent analysis of the cell and fibre architecture of the visual cortex in the rabbit (O'Leary & Bishop, 1938) indicates that the afferent fibres of the optic radiation terminate (at least mainly) in the lamina granularis interna, and the deeper part of the lamina pyramidalis. But whatever may be the precise site of termination of afferent fibres in the cell laminae of the visual cortex, it remains true that at each single point in the surface extent of the cortex impulses arrive from corresponding points of both retinae. Hence the cortical mechanism for the fusion of images necessary for stereoscopic vision is extremely localized spatially. Further, if the speculation advanced above is valid—that the three laminae in the lateral geniculate body which receive fibres from each retina are related to the three fundamental colour sensations postulated by the theory of trichromasy—then the projection of corresponding cells in each lamina to a single point in the visual cortex also provides for the immediate fusion of impulses from any point in the field of vision which is necessary for the recognition of colour mixtures.

If the evidence based on the Marchi experiments recorded in this paper, as well as on the observations of strychninization of the cortex, is accepted, it

[1] In a recent study on the electrical activity following optic nerve stimulation in the cat, Bishop & O'Leary (1940) led off from the geniculate body of one side while stimulating both optic nerves with single shocks. No evidence was obtained of any phenomena of interference or facilitation which might suggest fusion of retinal images at the geniculate level.

follows that there are no direct fibre-tracts whereby the retinal impulses impinging on the striate area can immediately be projected thence to distant cortical areas in the parietal or temporal lobes, to serve as a basis for visuo-tactile or visuo-auditory associations. Even within the striate area itself there appears to be no mechanism whereby a newly arrived retinal impulse can be directly diffused throughout the whole visuo-sensory cortex. On the contrary, the anatomical data (so far as they are at present known) indicate that, where it involves neurones whose axones run in the subcortical white matter, such a diffusion can only take place by relays of short neurones, and that by means of these each activated spot on the cortex can spread its influence over a radius of not more than 5 mm. or so. It must be added that neuronal activity in the cortex can presumably also be diffused by purely intracortical connexions, possibly by way of the fibres of the stria of Gennari which (in the monkey's brain) is predominantly an intrinsic fibre system of the visual cortex (Clark & Sunderland, 1939).

There can be no doubt that an activation of the visual cortex must in some manner be capable of influencing the physiological state of distant areas of the cortex to provide for the coordination of the whole cerebral mechanism which is necessary for complicated visual reactions. But it appears that this can only be effected, in the first instance, by means of short relays of neurones to the immediately adjacent areas; from the latter, longer association paths (such as those whose existence has been indicated by the experiments of Dusser de Barenne & McCulloch) may allow for the further spread of activity to more distant parts of the cortex.

SUMMARY

In three experimental lesions in the visual cortex of the monkey, the examination of Marchi material failed to show the existence of long association fibres leading to other parts of the visual cortex, or to the cortex in front of the lunate sulcus. These negative results are in accordance with the results of recent electro-physiological studies of the cortex. In one of the experiments there was an indication of association fibres leading from the visual cortex to a narrow strip of peristriate cortex lying under cover of the occipital operculum. The organization of the visual cortex in relation to the projection of retinal impulses is discussed.

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THE DENTAL COMB OF LEMURS

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Although the use of the procumbent anterior teeth of the mandible in lemurs in combing the fur has been affirmed by most workers from Cuvier & St Hilaire (1829) onwards to Wood Jones (1929), Zuckerman (1933), and Le Gros Clark (1934), this has been recently called to question. In a paper entitled "The myth of the lemur's comb", Stein (1936) denies that the teeth are so used, and claims to have exposed an error which has been perpetuated in the literature for over a century. It is suggested, indeed, that subsequent authors have relied entirely on the statement of Cuvier & St Hilaire without troubling to verify it by personal observation. Stein's conclusions seem to rest on two lines of inquiry. It is stated, in the first place, that in dead specimens the procumbent teeth are in such close contact with each other that "it requires considerable pressure to pass the finest waxed thread" between them. This curiously artificial observation leads the author to assert "the utter impossibility of a combing apparatus" in the disposition of the teeth. Such a conclusion, however, seems to be based on the assumption that the fur of a lemur's coat is comparable in texture with waxed threads! Stein also made a few observations on living lemurs, but it is admitted that conditions for such observations were most difficult. However, the fact that the intervals between the teeth did not contain hairs in three *dead* animals was taken as conclusive evidence that the teeth are not used as a comb. It appears obvious that the rapid cleaning of the teeth with the sublingua during life would almost certainly have removed any traces of fur which may have been caught in the teeth after grooming, and it is equally clear that this fact has been overlooked by the author. In any case, it would seem desirable to make a more careful examination of the matter before so lightly discarding a generally accepted statement based on the observations of a number of zoologists and naturalists of repute.

The present paper records observations which were made at the Zoological Gardens in London. Eleven individuals of the three species of lemur, *L. catta*, *L. macao*, and *L. fulvus*, were studied. The ring-tailed lemurs (*L. catta*) were the easiest to observe, and it was often possible to study their behaviour closely at a distance of only 18 in. from the front of the cage. Cinematographic films were taken, partly in the sunlight, and partly in the enclosed section of the cage with the aid of five 250 W. Photoflood lamps.

OBSERVATIONS

When they were not eating or sleeping, the animals spent a very considerable part of their time in grooming their fur, particularly while basking in the

sun or in the rays of the Photoflood lamps. For grooming, the tongue, the teeth, and the hindlimbs are all employed.

The tongue is used for licking the fur in much the same way as it is by cats, and, although this point was not closely investigated, the animals generally lick in the direction of the hair tracts. The tongue-licking alternates with a definite combing movement of the lower procumbent teeth. In this action, it is very important to note that the mouth is opened, for this precludes the possibility that the teeth are simply used in nibbling or biting. On the contrary, the lower teeth can be seen to be drawn over the skin, pressing down on the fur as they do so. The animals also go through an action of biting, and in this case short sharp movements are made by the lower jaw, in the same way that a cat bites at a tangle in its fur. Immediately after grooming, the inside of the mouth of a brown lemur (*L. fulvus*) was examined, and each lower front tooth was seen to be surrounded by a small mat of hair, which had collected in the intervals between the teeth during the combing movements and the biting action (Pl. 1, figs. 1, 2).

The feet are used for scratching, and the relative length of the hindlimbs allows them to reach almost any part of the body. As is well known, the second digit bears a "claw" which contrasts with the flattened nails of the other digits in being more projecting and compressed. In the brown lemur, the claw is somewhat more differentiated than in the other species which were studied. The hands are used in fixing the part to be groomed so that the mouth can approach it.

On several occasions, brown lemurs were seen to extend and withdraw their tongues rapidly for half a minute or so at a time, a movement which was quite distinct from the action of cleaning the muzzle. This movement probably represents the cleaning of the lower front teeth with the extremely well-developed sublingua, though it was not possible to make certain of this.

Much of the grooming performed is mutual, and (at least in *L. fulvus* and *L. macao*) this was irrespective of sex, or even of species. Inaccessible parts such as the face and the top of the spine are groomed by another individual, the two animals often operating on one another at the same time.

DISCUSSION AND CONCLUSIONS

My observations have shown that the procumbent front teeth of lemurs are by no means too close together to be used for a comb, as Stein has maintained. Even if they touch at their base, the points are sufficiently separated to allow the fine hair of the lemur to pass between them; indeed, the presence of hair round the individual teeth after grooming demonstrates this point conclusively.

Stein's statement that lemurs spend only a few seconds each hour in attending to the fur was not borne out by the results of the present investigation. On the contrary, under favourable conditions, the greater part of their waking hours was spent in grooming. It may be inferred, therefore, that Stein's

observations on living lemurs were made without due attention to the animals' surroundings.

Lastly, Stein claims that the procumbent lower teeth are modified in adaptation to the frugivorous habits of the lemur. However, since the upper incisors are much reduced, it is difficult to see how the lower teeth can be used in eating, as there is very little for them to bite against. In fact, it was actually observed that all three species which were studied always bite their food from the first with their side teeth (Pl. 1, fig. 4).

SUMMARY

The modified incisors and canines of lemurs are used as a comb, as is generally supposed to be the case. They are assisted in grooming by the use of the tongue and the digital claw of the hindlimb.

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EXPLANATION OF PLATE 1

Fig. 1. The mouth of a brown lemur (*L. fulvus*) opened to show the teeth. The hair can be seen to be lodged between the lower incisors just above the lower match-stick.

Fig. 2. The mouth of a brown lemur opened to show the teeth. A small mat of hair is seen lodged between the incisors and lying across the lower match-stick.

Fig. 3. Ring-tailed lemurs showing mutual grooming. The lemur to the right is using the combing motion, while the other is licking.

Fig. 4. Ring-tailed lemurs showing the method of eating, and showing that the food is inserted at the *side* of the mouth.

Fig. 2.

Fig. 4.

Fig. 1.

Fig. 3.

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THE DIFFERENTIATION OF THE BRAIN AND OTHER TISSUES IN AN IMPLANTED PORTION OF AN EMBRYONIC HEAD

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In a recent communication it has been shown by Le Gros Clark (1940) that fragments of the cerebral cortex of a foetal rabbit, when implanted in the brain of a young animal, not only survive but are capable of continuing their development. Embryos of between 15 and 18 days were used in his experiments. The foetal brain was freed of its mesodermal covering, and portions of the cortical layer of the cerebral hemisphere were placed in position in the host brain with the use of a modified sternal puncture needle. The results obtained from these experiments indicated that the neuroblasts of the foetal cortex have the capacity to continue their development in the host brain to form mature nerve cells. The latter showed all the structural characteristics of normal cells, including the Nissl-granule content and the usual appearance of neurofibrillae. It was thus evident that the neuroblasts are capable of differentiation in the implant, independently of their normal connexions. Furthermore, it was shown that nerve cells of similar morphological type had a distinct tendency to organize themselves in layers, with their cell processes, and particularly the apical dendrites, orientated more or less in a common direction.

These observations raise the question whether grafts, not only of small fragments, but also of larger portions of the central nervous system, can be successfully made with a view to studying their developmental potencies. With this object, further experiments have been attempted by implanting the *anlage* of the cerebellum, rhombencephalon, thalamus, hypothalamus and cerebral hemispheres of 18-day rabbit embryos. These were not successful, though the reason for their failure is not apparent. It may be mentioned, however, that in contrast with the technique employed by Le Gros Clark in his experiments, a wider operative exposure was made of the host brain with a view to obtaining more accurate implantation, the lateral ventricle being opened with the help of diathermy. Possibly this more elaborate method may have introduced disturbing factors which militated against the survival of the grafts.

In another experiment, a considerably younger embryo was used, namely 12 days, and in this case an implantation was made of the whole head as far back as approximately the level of the middle of the rhombencephalon. The result was remarkably successful, for the graft not only survived but underwent quite extensive differentiation within the host brain. The present communication is a report of this interesting specimen.

METHODS

Immediately before implantation the embryonic fragment was placed in a drop of plasma, and the latter then coagulated by the addition of a few drops of embryonic extract. It was hoped, by this procedure, (1) to provide for the nourishment of the implant until it should be reached by the blood vessels of the host brain, and (2) to minimize any possible injury to it during the necessary manipulations. Instead of introducing the implant into the substance

Text-fig. 1. Horizontal section through the head region of a rabbit embryo of 12 days, taken from the same litter as the implanted tissue. The section shows the diencephalon with the optic diverticula, and the rhombencephalon. ×55.

of the host brain as in the previous experiments, it was placed in position with a spatula on the pial covering of the cerebral hemisphere. The host rabbit was killed 24 days after the operation. Exposure of the site of implantation brought to view a rounded and circumscribed tumour, lying on the surface of the left cerebral hemisphere, and covered with pial tissue. The tumour measured about 1 cm. in diameter, and was well encapsulated. After removal it was cut in half, each part embedded in paraffin, and serially sectioned. Alternate sections were stained with gallocyanin and Bodian's protargol technique.

For comparison with the differentiated tissues seen in the graft, a brief reference may be made to the stage of development seen in a control embryo from the same litter, of which a transverse section is shown in Text-fig. 1. The primitive neural tube has already differentiated into the five main parts, the forebrain, diencephalon, midbrain, rhombencephalon and myelencephalon. From the diencephalon the outgrowth of the optic primordium has appeared, and the optic vesicle is beginning to undergo its invagination to form the inner and outer layers of the optic cup. The ectoderm overlying the cup is becoming thickened to form the primordium of the lens. The rhombencephalon shows a sulcus medianus internus rhombencephali, and the beginning of the sulcus lateralis. Below the lateral part of the floor of the rhombencephalon the *anlage* of the cranial nerve ganglia are discernible. The ependymal epithelium of the ventricular system is not yet differentiated, nor have the chorioid plexuses yet begun their development.

The otic vesicle at this stage is beginning to elongate and shows an indication of the main parts of the internal ear—the cochlear and vestibular parts and the commencement of the endolymphatic diverticulum.

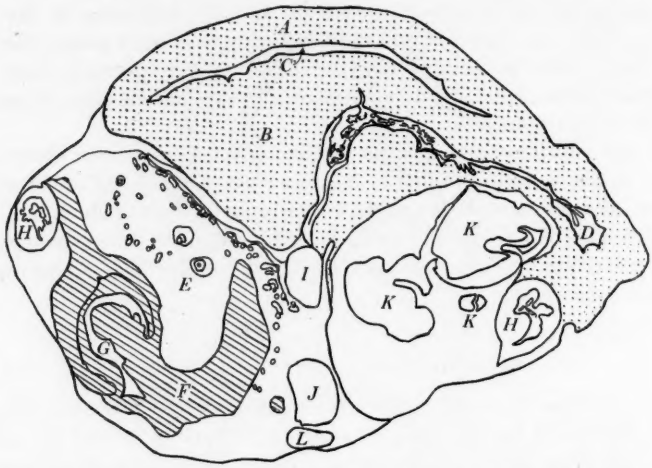
In this stage of development the wall of the cerebral vesicles is histologically differentiated into a zona ventricularis with ventricular mitosis, a zona nuclearis and a zona marginalis.

HISTOLOGICAL OBSERVATIONS

A general view of a section taken through the middle of the implant shows a differentiation of the following structures (see Text-fig. 2, and Pl. 1, fig. 1). Rather more than half of the tumour is composed of nervous tissue associated with which there can be identified a layer of skin containing hair follicles with well-developed hairs, and a mass of cartilage in the middle of which is a tubular passage lined by ciliated epithelium. The other half of the tumour is made up of tooth germs and epithelial structures. Towards the centre of the whole mass a differentiated lens is present, together with a portion of the pigment layer of the eye and lacrimal gland tissue, and also part of the semicircular canal system. Other tissues, such as bone, mucous glands and striated muscle, are also very evident in some sections, but while they are readily recognizable histologically, their appearance and topographical relations do not in general allow them to be identified with any particular morphological element of the normal animal. It is of interest to note that the different elements which compose the tumour show very contrasted growth tendencies. While, for example, the muscular tissue is still embryonic in character, and the eye is small, ill-formed and rudimentary, the tooth germs have proceeded very far in their growth and differentiation, and in relative size they far exceed most of the other elements. Indeed, in the extent of their development, they appear to approximate closely to the normal tooth germs of a new-born rabbit. The hairs also have reached a remarkable maturity, and in some cases have grown far into

the neighbouring tissues. The contrast shown by the rapid development of the epidermal derivatives calls to mind the observation that the same tissues continue to grow at almost their normal rates in young animals even when the growth of most other tissues has been notably retarded or completely arrested by gross dietary deficiencies.

A consideration of the external form of the nervous tissue, as seen in some of the sections, makes it apparent that it represents an attempt at the production of bilaterally symmetrical structures, and a more detailed study shows



Text-fig. 2. Schematic section approximately through the middle of the graft (cf. Pl. 1, fig. 1). The nervous tissue is indicated by the stippled area, the ventricular cavities being outlined by a continuous line. Cartilage is represented by hatching. *A*, surface layer of cortical tissue; *B*, wedge-shaped mass of nervous tissue in which the nerve cells are partly arranged in a laminar formation, and partly collected into circumscribed groups resembling "nuclei"; *C*, *D*, ventricular cavities; in the cavity on the right there is a well-developed tela chorioidea; *E*, skin, with hair follicles and hairs; *F*, cartilage, the margin of which is undergoing ossification; *G*, tubular formation lined by ciliated epithelium; *H*, tubular formation lined by stratified epithelium; *I*, tubular formation lined by columnar epithelium; *J*, lens; *K*, tooth germs; *L*, small mass of bone.

that these are rudimentary cerebral hemispheres. A section through the middle of the mass shows that they are each composed of a surface layer (Text-fig. 2, *A*) overlying a deeper wedge-shaped formation (*B*). The two parts are separated by a well-differentiated ventricular cavity, but at the lateral margin of the latter they become continuous.

At a higher magnification the surface layer of nervous tissue shows all the structural characteristics of a cerebral cortex. The nerve cells are arranged in definite layers, and it is possible to distinguish small and large pyramidal cells, as well as cells of the granular type. In the gallocyanin sections, the pyramidal

cells are seen to have a normal arrangement of Nissl granules, and the protargol sections demonstrate the presence of a neurofibrillary structure. The apical dendrites of the small and large pyramidal cells are, in general, orientated in a common direction.

The basal wedge-shaped part of the nervous tissue shows in the neighbourhood of the ventricular cavity a rather characteristic structure. Large pyramidal cells have become arranged in a narrow compact layer, and lie in immediate relation to a parallel row of small granular cells (Pl. 1, fig. 3). The pyramidal cells have a large nucleus, a broad base and relatively short apical dendrites. The whole formation bears a general resemblance in its cyto-architecture to the hippocampal cortex of a normal rabbit's brain. Moreover, a tract of fibres takes origin from it, and this can be followed in serial sections across the median plane to a corresponding structure on the opposite side of the whole mass of nervous tissue. Indeed, we are led to infer that this is a commissural bundle, probably representative of the fornix commissure. Not all parts of the nervous tissue show a more or less distinct cortical structure. In the basal portion are found areas where ganglion cells are arranged in definite groups or "nuclei", and sharply separated by cell-free zones from neighbouring regions. The cells which form such circumscribed groups are almost always of a common type. Thus, some groups consist predominantly of large ganglion cells, and in others only small cells are present.

Clear-cut tracts of fibres can be followed from the cortical formations as well as from the nuclear groups (Pl. 1, fig. 2). Attempts have been made to trace these tracts in the serial sections, but it has not been possible to homologize them with recognizable fibre paths of the normal brain. However, it is possible to state that the cells which take on a laminar formation (distinctive of a cortical structure) send out fibres which go partly to the more deeply situated ganglion cells of the lamina, and partly to more distant parts of the nervous tissue. Further, the cortical formations are also connected by fibre tracts with the more deeply situated nuclear groups of cells, and it is tempting to see in this arrangement an attempt at the development of thalamocortical radiations if we can suppose that the nuclear groups represent thalamic nuclei. In any case, however, it is interesting to note that the several parts of the mass of nervous tissue in the graft have become interconnected by fibres which are collected into definite and well-circumscribed fasciculi.

The ventricular septum is lined by flat cuboidal epithelium, and at certain points the epithelium is invaginated to form quite typical chorioid plexuses within the folds of which numerous blood vessels are present. The differentiated mass of nervous tissue, therefore, has its own closed ventricular system with all the apparatus for the secretion of cerebrospinal fluid.

It is to be noted that the neural tissue of the central nervous system which has undergone differentiation in the implant appears all to be referable to the higher functional levels of the cerebral hemispheres, and most of it is clearly derived from cortical elements. On the other hand, the part which shows an

arrangement of "nuclei" seems to represent a thalamic development. This inference is based not only on its cytoarchitectural organization, but also on the fibre connexions which it has established with the differentiated cortex. Nowhere was it possible to detect a neural organization suggestive of lower functional levels of the brain, such as the basal ganglia, midbrain, pons and cerebellum, even though the embryonic rudiments of these structures were also included in the graft.

A further study of the sections shows that parts of the peripheral nervous system have also undergone development in the graft. At the base of the main mass of nervous tissue, between it and a mass of developing bone, is an elongated ganglion of nerve cells (Pl. 1, fig. 4). The latter are round or pyriform, each with a vesicular nucleus often containing two nucleoli. Each cell emits one cell process only, and this neurite typically makes a spiral turn around the cell before it leaves the ganglion. All the processes are collected into one bundle, and this runs into the neighbouring connective tissue and muscular tissue. The structure has all the appearance of a somatic sensory ganglion, but it was not possible to detect any T-shaped bifurcation of the axone at its commencement, nor were any nerve endings observed in relation to the muscle fibres. Another ganglion of quite a different type was found embedded in connective tissue and lying in a deep depression in the mass of bone. Here the ganglion cells are multipolar and very large, and the structure has all the appearance of an autonomic ganglion. The axones of these cells can be traced in among neighbouring bundles of striated muscle and also into glandular tissue of mucous type. The nerve fibres which enter the muscle tissue branch among the muscle fibres, and give the impression of actually forming small end bulbs on the latter. Those which enter the gland tissue ramify among alveoli, but it was not possible to identify end bulbs.

Striated muscle tissue has undergone a very prolific development in the graft. Each fibre shows the characteristic striation, and they are arranged either in coarse bundles or in diffusely growing strands. Their growth seems to be quite unrestrained, so that they tend to fill up any gap not occupied by other tissues. In several places muscle fibres actually penetrate into the substance of the nervous system. In the silver-impregnated sections a rich plexus of nerve fibres can be seen in the muscular tissue; where the latter penetrates into the nervous tissue, these fibres apparently take origin directly from neighbouring nerve cells. The fibres are provided with a sheath of neurilemma cells. There can be little doubt of the specific affinity between the nerve fibres and the muscular tissue during the process of differentiation, for in the neighbouring connective tissue only very few nerve fibres are to be found.

The development of the eye and semicircular canals in the graft show some points of interest. The inner and outer layers of the optic cup have undergone considerable differentiation. All the layers of the retina are recognizable and the pigment layer shows a great concentration of pigment granules (Text-fig. 3). Some vestiges of the vitreous body can be detected and the lens is also present,

but situated at some distance from the other parts of the eye. No optic nerve, or nerve fibres from the ganglion layer of the retina, could be definitely observed.

Text-fig. 3. The retina and pigment layer of the eye in the implant. ×60.

Text-fig. 4. Section showing semicircular canals. ×27.

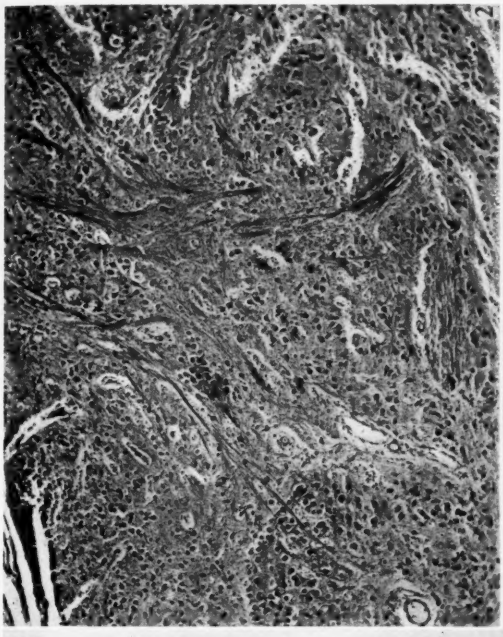
Inside a mass of cartilage, the margins of which are already undergoing ossification, is seen a canal system which bears all the characters of the semicircular canals, but no nerve fibres could be detected in relation to them (Text-fig. 4).

The other formations, such as hairs, skin, mucous glands, bone and connective tissue, show a histological differentiation corresponding to that of a normal animal and call for no special remarks. The rudiment of the tongue, however, merits a brief reference. It consists almost entirely of a surface covering of epidermis with quite typical papilla formation, and enclosing connective tissue and mucous glandular tissue. The musculature has not grown into the organ, but, in spite of this, the latter is definitely recognizable as a part of the tongue. The absence of its musculature provides support for the thesis that the tongue muscles are derived from a forward migration of occipital myotomes, for the latter were not included in the part of the embryonic head which was implanted. On the other hand if, as some authorities believe, the tongue musculature is normally developed as a local differentiation of the mesoderm in the ventral wall of the pharynx, it seems probable that it would have undergone development in the graft for, as already noted, there is plentiful striated muscular tissue elsewhere.

DISCUSSION

The foregoing experiment demonstrates that embryonic cortical tissue possesses a considerable capacity for self-differentiation. This capacity is expressed in the development of individual neuroblasts to form mature cells typical of those found in the normal cerebral cortex, with a normal appearance of Nissl granules and neurofibrillae. Moreover, the cells become disposed in layers which are similar in their arrangement to the lamination of normal cortex. Indeed, in one part of the graft the cytoarchitectural features are sufficiently characteristic to allow the provisional identification of a hippocampal cortex. It is also to be observed that the structural organization of the cortex is maintained even in the absence of all afferent fibre systems such as the optic nerves, the olfactory nerves and the fibre tracts of the spinal cord. The independent growth and differentiation of the cortex find further expression in the fact that its various parts are not connected together by a diffuse unorientated network of fibres, but by individual, well-circumscribed fasciculi. The whole process suggests, indeed, that the cerebrum is, in itself, an independent developmental unit, and that even its component parts may be regarded as separate morphological entities (*topische Einheit* of Vogt).

It is proposed to carry out further experiments to determine whether other parts, such as the midbrain, cerebellum and rhombencephalon, are capable of independent differentiation. The lack of success which has hitherto attended such grafts in this laboratory may be the result of inadequate technique. On the other hand, the results of the present experiment, together with a survey of the previous experiments, have given the impression that while cortical tissue can undergo quite extensive differentiation in implanted fragments, lower levels of the brain have not the same capacity. This gives rise to the interesting supposition that in the central nervous system the capacity for



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independent differentiation is limited to the more highly organized functional levels. But this is a matter which can only be settled by a relatively large series of experiments.

SUMMARY

An account is given of the differentiation of tissues in a portion of the head of a 12-day rabbit embryo, implanted in the brain of a young rabbit. The differentiation of nervous tissue had proceeded to the formation of a definite cortical structure with a laminar arrangement of ganglion cells, of groups of cells showing a nuclear arrangement, and of circumscribed fibre tracts.

The author wishes to express his thanks for the assistance of a grant from the Nuffield Committee for the Advancement of Medicine.

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EXPLANATION OF PLATE 1

Fig. 1. Section through the implant, corresponding to the schematic diagram shown in Text-fig. 2. ×11.

Fig. 2. Part of a section through the nervous tissue (stained with Bodian's protargol technique) showing the nerve cells arranged in a series of "nuclei", and also the well-defined fasciculi of nerve fibres which interconnect different nuclear and cortical elements. ×69.

Fig. 3. Part of a section through the nervous tissue (stained with gallocyanin) showing the laminar arrangement of granule cells, small and large pyramidal cells. ×72.

Fig. 4. Section through a peripheral sensory ganglion, stained with Bodian's protargol technique. ×114.

TENSION LINES, CLEAVAGE LINES AND HAIR TRACTS IN MAN

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Manchester

In 1816 J. H. Cloquet, in describing the surface of the skin, noted the various lines produced by muscle action and by stasis areas over joints: but he also called attention to other lines that were not so caused. These lines were finer and less conspicuous than the familiar crease lines, and Cloquet supposed that they were caused "par une disposition particulière du tissu cellulaire", and he especially instanced the fine lines on the neck as being outstanding examples of such a causation.

In 1825, Monro tertius also noted that there were fine wrinkles on the skin not occasioned by the action of muscles under the skin. Subsequent writers on the skin, even those who have dealt with it as their professional preoccupation, seem to have taken but little notice of the fine patterning that is everywhere evident in the texture of the skin. The work of Oscar Simon in 1873 appears to have attracted but little attention, and with but few exceptions (such as Kaposi) skin specialists have failed to mention it in their writings. Simon's charts of these wrinkles—"Oberhautfurchen"—were published as Figs. 3 and 4, Pl. V, in his monograph on *The Localization of Diseases of the Skin*. These charts are reproduced here as Figs. 1A and 2A. There seems to be no reason to doubt the explanation for the presence of these lines, given originally by Cloquet and elaborated by Simon, that they are the expression of the disposition of fibrous tissue in the skin. Nor is there any reason to reject the thesis of their causation implied in the term "tension lines" of the skin.

In 1834 Dupuytren, in describing wounds of the skin made by penetrating instruments, gave details of the case of a cobbler who committed suicide by stabbing himself with his awl. The awl was pointed at the tip and was round in section. But the wounds on the cobbler's skin were not rounded but linear in outline, and appeared to have been made by the blade of a knife. Dupuytren's assistant at the time was M. Filhol, and he carried out a series of experiments that showed quite conclusively that it was impossible to make a round hole in the human skin by piercing it with a sharp instrument of circular section. In 1838 Malgaigne gave an excellent account of the whole matter in his text-book on *Surgical Anatomy and Experimental Surgery*. He considerably extended Filhol's researches and showed that there was a constancy in the direction of the slits made in any definite region of the body. It was Langer who made the facts concerning the "Spaltbarkeitslinien"—the cleavage lines of the skin—available to students by publishing his charts in 1861–2. More recently, Dr H. T. Cox of Manchester has repeated Langer's work on a far

more extensive scale, and his results confirm, in most instances, the findings of the pioneer workers. Langer's charts are reproduced here as Figs. 1B and 2B.

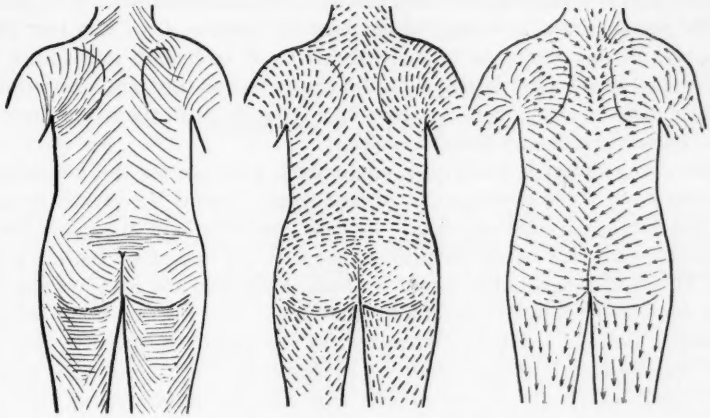

Fig. 1. Dorsal surface of the trunk. A, Simon's "Oberhautfurchen"; B, Langer's "Spaltbarkeitslinien"; C, hair tracts.

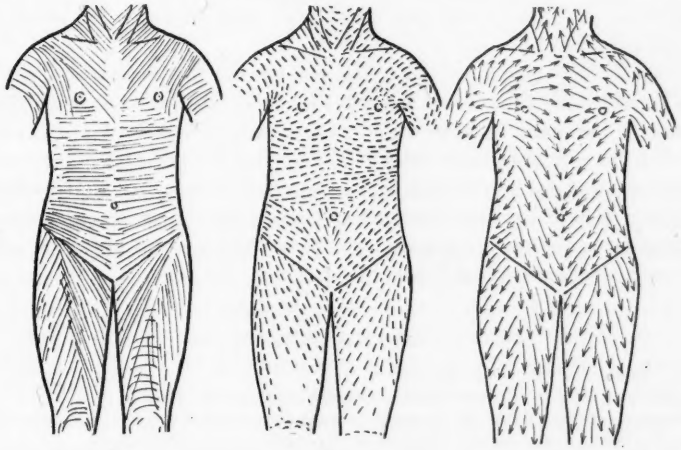

Fig. 2. Ventral surface of the trunk. A, Simon's "Oberhautfurchen"; B, Langer's "Spaltbarkeitslinien"; C, hair tracts.

When Simon had completed his work of charting the fine wrinkles of the skin it was at once apparent that his figures were practically identical with those made ten years previously by Langer. There can be little doubt that the

tension lines of Simon are the surface manifestation of the cleavage lines of Langer, and that they are caused by the disposition of the strands of fibrous tissue in the skin. This fact can be dramatically demonstrated by cutting sections of skin parallel to the lines and at right angles to them.

The tension lines are so easy to see upon the surface of the skin that there can never be any doubt as to their direction. It is enough to instance the longitudinal lines on the palmar aspect of the digits and the transverse wrinkles on their dorsal aspect. Nor can there be any doubt that the name "tension lines" correctly indicates their causation.

Here no attempt is made to produce fresh evidence for the Lamarckian interpretation of the development of hair tracts. But it is relevant to point out that the theory that appears to be most generally held in explanation of the well-known hair trends postulates that they are caused by tension in the skin. This is the explanation urged by Bolk, Voigt and the many others who have completely rejected the thesis that they may be caused by outside influences, such as habit, the operations of the toilet, and even by gravity. Were the disposition of hair tracts to be determined by the internal tension in the skin we should expect to find that Simon's charts should not only strongly resemble those of Langer but that they should also resemble the charts of hair tracts that have been produced by so many observers (see Figs. 1C and 2C). It is at once apparent that, except for some occasional general similarities, the hair-tract charts differ profoundly from those of the tension lines. In many cases hair trend is at right angles to the direction of the tension lines.

Here only the tracts on the trunk are figured for comparison with the charts produced by Simon and Langer, and there is no need to draw especial attention to the many areas in which wide discrepancy is displayed. It would seem that there must be some other factor beyond the presence of mere strains and stresses in the skin involved in the causation of hair tracts. The orthodox alternative to a more Lamarckian explanation of the trends of body hair, including reversals, partings and convergences, appears to lack any support when tested by the work of Simon and Langer.

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THE DISTRIBUTION OF ASCORBIC ACID (VITAMIN C) IN THE EARLY STAGES OF THE DEVELOPING CHICK EMBRYO

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1. INTRODUCTION

In 1928 Szent-Györgyi published the results of researches on a strongly reducing substance in the cortex of the mammalian adrenal. This substance, which was then called hexuronic acid, was found to have reducing properties which exceed those of other known intracellular reducing agents: in particular, it is capable of reducing silver nitrate in the dark with great rapidity. Later, Harris & Ray (1933*b*), among other authors, showed that the substance is identical with the antiscorbutic vitamin C; the vitamin was therefore renamed ascorbic acid. On the basis of these studies a method has been elaborated, mainly by Bourne (1933*a*, *b*, *c*), Leblond (1934) and Giroud (1938), by which ascorbic acid can be specifically identified in histological preparations. The method has been applied to extensive investigations of the adult tissues of various species (see Giroud, 1938), but little use has hitherto been made of it in the study of embryonic cells and tissues. A survey has therefore been made of the distribution of ascorbic acid in developing chick embryos. The findings of this survey, in embryos up to the fourth day of incubation, are reported below.

2. METHODS

Silver nitrate is used by histologists in several ways. The method of Cajal for the staining of nervous tissue involves the fixation of the salt by the tissues, and its later reduction by a further reagent such as hydroquinone; this is also the case in Da Fano's method for Golgi substance. By contrast, the use of silver nitrate for the demonstration of ascorbic acid depends on direct reduction: in this case the *p*H of the silver nitrate solution becomes of primary importance. Silver nitrate in ammoniacal solution is very easily reduced, in neutral solution much less easily, whereas in acid solution it is reduced with comparative difficulty: thus according to Mellor (1923) it undergoes no reduction in solution with organic acids such as malic, tartaric, citric and malonic, even when boiled, unless there is an oxidising agent such as potassium permanganate present. There are inorganic substances, such as hypophosphorous and phosphorous acids, which reduce silver nitrate with the production of a black precipitate, but they are not of a kind which could exist free in living cells.

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In considering organic reducing agents it is important that the acid silver nitrate method for the demonstration of ascorbic acid involves the use of the reagent in definite physical conditions: it is used only in the cold, in the dark, and over short periods of time. In these conditions it is known that other strongly reducing substances which occur in cells have no effect on the reagent. Leblond (1934) has shown this to be the case for adrenalin, polyphenols such as tannin, various sugars and cysteine. Bourne (1936) removed the lipoids from cells, by means of chloroform, before subjecting them to the reagent, and found that the reaction persisted. On the other hand, washing with distilled water or any aqueous solution results in the disappearance of the reaction, owing to the removal of the vitamin in solution. Leblond (1934) also used methyl alcohol, the best solvent for ascorbic acid, in order to demonstrate the same effect: washing small pieces of tissue in methyl alcohol for 10 min. results in an alteration of the reaction; the reducing substance can be seen to have diffused through the tissue as a result of the mobilizing action of the solvent. Exposure to the solvent for longer periods causes the complete disappearance of the reaction.

There are two certain methods for the estimation of the amount of ascorbic acid present in a tissue: the first of these is investigation of its antiscorbutic activity, and necessarily does not give accurate results; the second is titration with dichlorophenolindophenol (Harris & Ray, 1933*a*). A number of studies has been made showing that histological findings regarding the presence of ascorbic acid parallel the estimates made by these methods. Leblond (1934) gives a table of both plant and animal tissues, including adrenal, corpus luteum, muscle, orange, cabbage and mushroom, in which this parallelism is illustrated. Further examples may be found in the work of Giroud (1938).

None of the above facts is conclusive. The most important evidence for the specificity of the silver nitrate reaction is derived from the studies of scorbutic animals (Leblond, 1934; Bourne, 1935*a*; Demole *et al.* 1935; Giroud & Leblond, 1936, 1937). Outside the primates only cavies are known to be susceptible to scurvy (i.e. to be unable to synthesize vitamin C), and the work has been done on them. In a variety of tissues, including the adrenal, the testis and the kidney, it has been found that the reaction with silver nitrate disappears progressively when the animal is put on a scorbutic diet. The results of an extensive study of the kidney were published by Giroud & Leblond in 1937. The reaction with silver nitrate was found to persist in the kidneys of rats on a scorbutic diet, but to disappear progressively from those of cavies on the same diet. Intravenous injection of 50 mg. ascorbic acid into scorbutic cavies resulted in the immediate reappearance of the reaction. Similar observations on a number of tissues had already (1936) been briefly reported on by these authors; they had also observed that injection of ascorbic acid into normal cavies caused the appearance of reactions in tissues, such as the liver and the epithelial cells of the intestine, which did not before show them.

It is known that certain melanin pigments constitute an exception to the

rule that only ascorbic acid reduces acid silver nitrate (Giroud & Leblond, 1936; Giroud, 1938, p. 22). The melanin granules in the Langerhans cells and the Malpighian layer of the skin show a reaction which persists both after extraction with methyl alcohol and during scurvy. These pigment granules are easily recognizable, and the reaction they give does not constitute an objection to the use of the reagent for demonstrating the presence of ascorbic acid in other tissues.

A further difficulty arises from the fact that the liver and adrenal medulla, both of which are known from biological and chemical tests to contain large quantities of ascorbic acid, show the reaction with silver nitrate only slightly or not at all (Bourne, 1933*c*; Galvao & Cardoso, 1934). Harris & Ray (1933*a*) have suggested that this is due to the presence of a substance which inhibits the oxidation of ascorbic acid. It is known that glutathione has a stabilizing action on ascorbic acid in vitro and in vivo (Borsook *et al.* 1937), and the question arose whether it might not be glutathione, or at least the presence of free sulphydryl, which was responsible for the supposed inhibition. Various authors (Emmerie, 1934; Svirbely, 1935; Giroud *et al.* 1936) have carried out experiments in vitro which show that glutathione can prevent the reduction of acid silver nitrate, in the relevant physical conditions, by ascorbic acid. The last-named authors report that the inhibitory effect becomes important when the concentration of glutathione reaches a level corresponding to one molecule to five of ascorbic acid. Giroud (1938, p. 34) concludes: "Ceci permet d'une part de supposer que des phenomènes d'inhibition puissent fréquemment jouer quand on réfléchit à la teneur relativement élevée en glutathione en divers tissus et d'autre part, oblige à envisager dans bien des cas processus d'activation pour expliquer des résultats positifs." This conclusion takes no account of the possibility that glutathione, even if it is the only inhibiting substance, may be present in some tissues in a state in which it is unable to exert its stabilizing action; it does, however, make clear that the mechanism underlying the failure of the reaction in liver and adrenal medulla is still far from fully established.

It remains to be pointed out that silver nitrate is only reduced by ascorbic acid itself,[1] and not by the vitamin in its reversibly oxidized form, dehydroascorbic acid. In all normal tissues which have been studied the latter occurs in a concentration only one-tenth to one-twentieth of that of the reduced vitamin (Giroud, 1938, p. 4). Bourne (1936) found that treatment of tissues with H_2S, which reduces dehydroascorbic acid before impregnation, has very little effect on the amount of silver precipitated.

As a result of these observations it has been generally concluded that the production of a black precipitate in tissues by the acid silver nitrate method indicates the presence of ascorbic acid (with the one exception which has been mentioned). The alternative possibility is that there occur, in some cells, reducing conditions which simulate the action of ascorbic acid on the reagent.

[1] The sodium salt of ascorbic acid (Roche), as prepared for injection, also gives a black precipitate with acid silver nitrate.

Although there is no evidence for this possibility, it cannot be wholly excluded for material to which the scurvy test cannot be applied. Nevertheless, for the following account it has been held justifiable to assume that the reactions observed are unlikely to be due to reducing substances other than ascorbic acid.

Details of method

The solubility of ascorbic acid makes it necessary to fix the tissues and to impregnate them with silver at the same time. Two mixtures were used for this purpose: the first was a 10 % solution of silver nitrate in 10 % acetic acid; the second was a saturated solution of silver nitrate in ethyl alcohol (5 parts), water (4 parts) and glacial acetic acid (1 part); the latter gives a concentration of silver nitrate of slightly less than 10 %. Both mixtures give good results, but with the second there is better fixation and rather better penetration. Lower concentrations of silver nitrate were tried, but were not found to be satisfactory. Embryos up to 24 hr. require only 15–20 min. in the mixture; larger embryos were given progressively longer, and at 4 days they were left in the fixative for up to 45 min. Fixation was carried out in the dark, after the embryo had been removed from the yolk and separated from its membranes. It was followed by thorough washing in distilled water, before dehydration and embedding in paraffin. All sections were cut at 5 μ, brought down to water and toned in very dilute gold chloride for from 4 to 10 min.; they were then left in sodium thiosulphate solution for a similar period, dehydrated, "cleared" in xylene and mounted in Canada balsam. The toning is not essential, but it removes the yellow-brown coloration of the tissues which tends to obscure the black granular precipitate of metallic silver.

Table 1

Unincubated	5
12 hr.	2
18 hr.	3
24 hr.	5
36 hr.	1
48 hr.	2
72 hr.	4
96 hr.	13

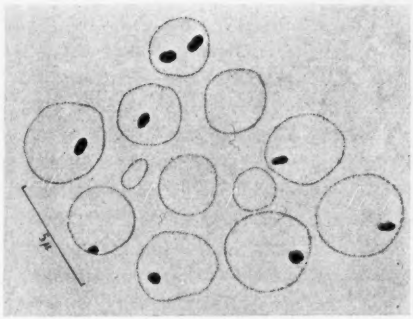
Table 1 shows the number of embryos used, with their ages. Embryos up to 24 hr. were first fixed *in situ* for 1 min. after removal of the albumen. This was done by holding over the yolk a piece of paper from which a small triangular section had been cut. A few drops of the fixative were allowed to come in contact with the embryo, which was in the uncovered, triangular area of the blastoderm. The fixed area was then cut out, with some yolk adhering to it, and left in the fixative for a further period of about 15 min. The shape of the triangle made possible the orientation of the embryo when it was embedded. Orientation in the first place was based on the relation of the blastoderm to

the poles of the egg. Embryos of from 1½ to 4 days were removed from the yolk with the whole blastoderm. The latter was floated out on distilled water, and held stretched by a small glass ring during fixation.

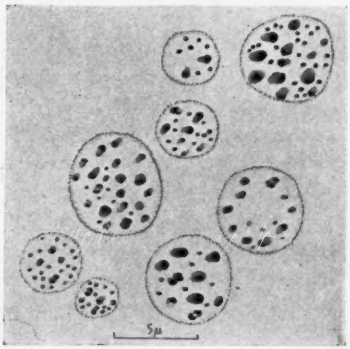
3. RESULTS

A. *Blastoderms before the appearance of an embryo*

Carrick & Hauge (1925) found that vitamin C is not present in the new-laid eggs of fowls kept on a scorbutic diet, but that its presence could be demonstrated in 5-day chicks hatched from such eggs. Ray (1934) was unable to find ascorbic acid in unincubated fowls' eggs by titration: titrable amounts appeared at the end of the fourth day of incubation, in the embryo but not in the yolk



Text-fig. 1. Extracellular yolk globules from unincubated egg. Small black deposits situated on a refractile granule (shown grey) represent ascorbic acid.

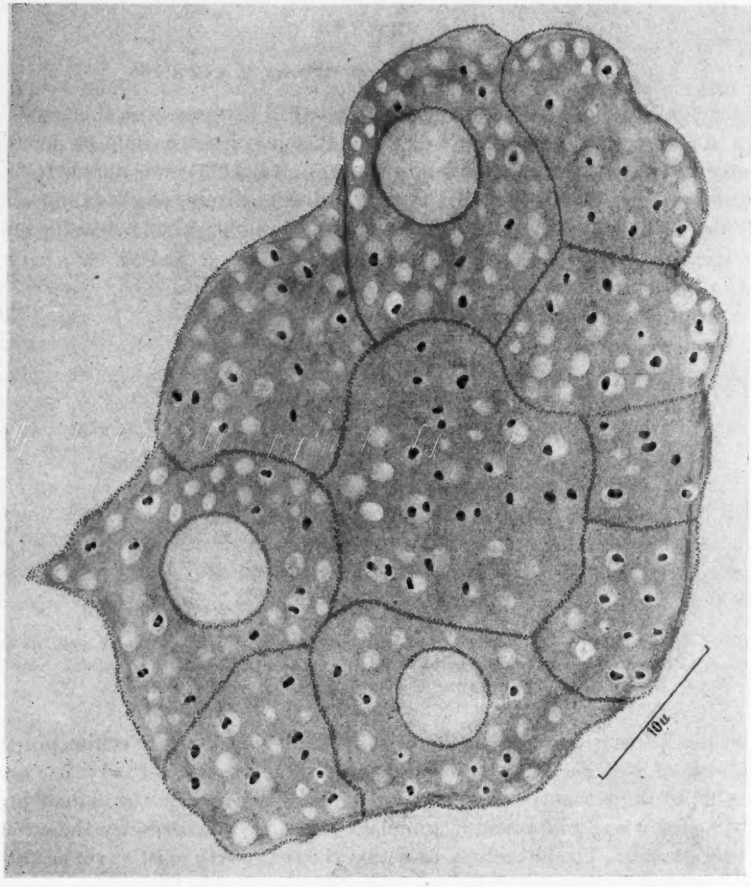


Text-fig. 2. Extracellular yolk globules from egg incubated for 18 hr. Note the greater number of granules.

or white. There is therefore no doubt that chick embryos early commence the synthesis of ascorbic acid. By using the acid silver nitrate method it has been possible to show that ascorbic acid has already appeared in the unincubated blastoderm, where it is present in granules of varying size attached to the surface of yolk granules. The latter may be within the cells of the blastoderm or in the layer of yolk which immediately underlies them; they may have several blackened granules attached to them, but generally at this stage they have only one (Text-figs. 1, 3, 8). It seems possible that the fact that ascorbic acid appears to be synthesized outside the blastoderm cells may be due to the diffusion of an enzyme from them into the yolk below.

By no means all the yolk granules have ascorbic acid in association with them. In the upper layer of the blastoderm (the epiblast) the black precipitate is confined to the periphery, and the remaining cells (which contain yolk) have no ascorbic acid. In the endoderm the diameter of the empty region is

smaller; in the layer of yolk granules below, the ascorbic acid may be evenly distributed, or it may be absent from a small central area (Text-fig. 8). It is clear that the region which is to form the area pellucida, and which will contain



Text-fig. 3. Endoderm cells from unincubated embryo. Small white globules represent intracellular yolk.

the primitive streak and the embryo, is in a different physiological state, with respect to ascorbic acid, from the rest of the blastoderm.

The small granules, attached to yolk globules, on which the silver is precipitated, frequently show irregular impregnations: often there are two black caps, crescentic in shape, at opposite poles of a granule, but there are many

variations (Text-figs. 1–3). It is not to be supposed that the exact form of the deposit is of great significance, since the precise arrangement of the molecules of precipitated silver will doubtless depend to a large extent on the physical conditions which prevail in the neighbourhood of the reducing agent.

At the 12th hour of incubation, i.e. at the stage at which the primitive groove appears, the distribution of ascorbic acid remains the same. At 18 hr., when the primitive streak reaches its greatest length, the black granules are confined to the yolk underlying the blastoderm. At this stage there is consequently no intracellular ascorbic acid; on the other hand the amounts associated with the yolk granules appear to be much greater (Text-fig. 2) than those in earlier blastoderms.

B. *Blastoderms after an embryo has developed*

Black deposits have reappeared in the cells by 24 hr. They are confined almost entirely to extra-embryonic tissues, and in particular to the ectoderm and endoderm. Owing to the unavoidably bad fixation obtained with the silver nitrate reagent, relatively few of the ectodermal and endodermal cells can be fully studied. Nevertheless, in the less distorted cells it can be clearly seen that the ascorbic acid is distributed in granules, spherical or rod-shaped, which occupy all regions of the cytoplasm: the cytological picture consequently resembles a mitochondrial impregnation (cf. Giroud, 1938, p. 26; Bourne, 1935*b*), the nucleus appearing as a clear space (Text-fig. 4).

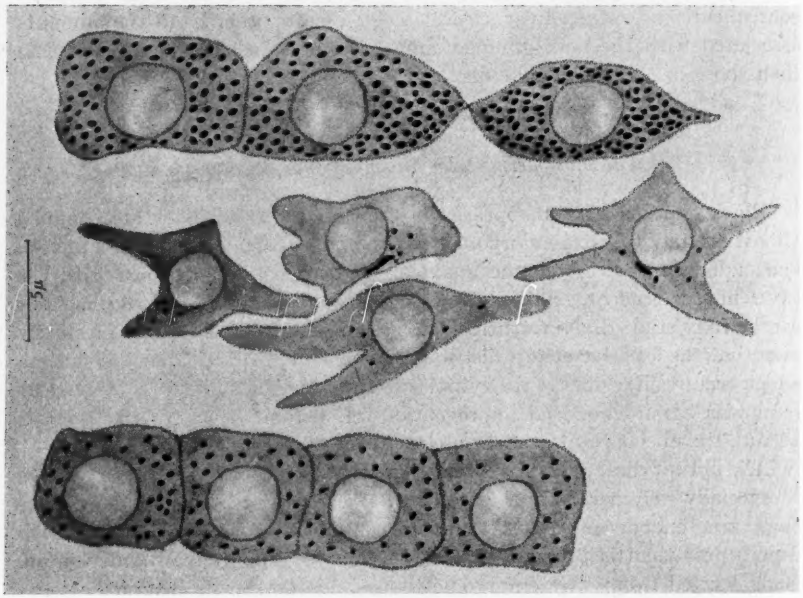
The abrupt disappearance of ascorbic acid at the boundary of the embryo is especially well marked in the ectoderm of embryos of from 1 to 3 days. The embryonic endoderm is often found to contain some ascorbic acid, though usually less than is present outside the embryo. The amnion and chorion, being formed from extra-embryonic tissues, contain heavy deposits. It has been found, however, that in 3-day embryos the deposit is absent from both layers immediately dorsal to the embryo. This phenomenon, which is not easily explained, may be compared with the absence of ascorbic acid from the yolk below the centre of some early blastoderms.

C. *The embryo after 4 days' incubation*

As we have seen, Ray (1934) was unable to find titrable amounts of ascorbic acid in chick embryos until the end of the fourth day of incubation. It is reasonable to suppose that the ascorbic acid demonstrated by the silver nitrate method in the early extra-embryonic tissues is present in too small quantity to be detected by the ordinary methods of quantitative analysis. At the fourth day, as might be expected from Ray's results, deposits of ascorbic acid appear

in a variety of tissues within the embryo. These include various parts of the central nervous system, part of the gut wall, some of the somitic material and certain mesenchymatous cells.

A striking and exceedingly constant phenomenon in the central nervous system is the dense impregnation of the dorsal ependymal layer at all levels (Pl. 1, fig. 11), and the heavy deposit which fills the thin roof-plate of the medulla; in the spinal cord, too, it is found that the inner, or ependymal, layer

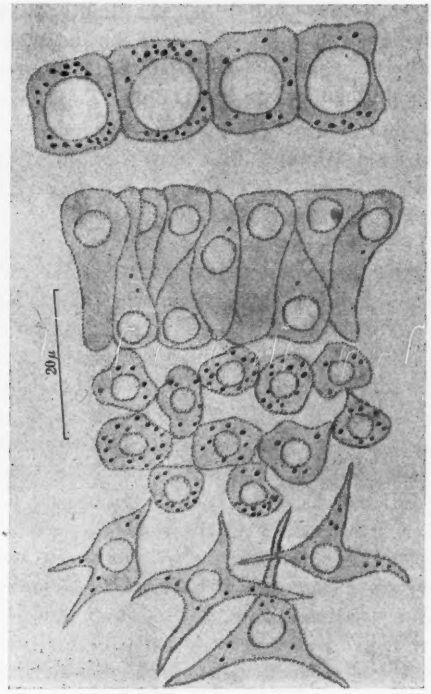


Text-fig. 4. Extra-embryonic cells of a 36 hr. embryo.

of the roof-plate is more heavily impregnated than other regions. The appearance of the medullary roof-plate is similar to that of the extra-embryonic ectoderm already described: that is to say, the cytoplasm of each cell is packed with spherical granules resembling mitochondria. On the other hand, the impregnation of the roof-plate in the spinal cord is unusual in being mainly on the cell membranes: relatively little of the black precipitate is within the cells (Pl. 1, fig. 12).

The other parts of the brain which, at this stage, contain conspicuous quantities of ascorbic acid, are the diencephalon and midbrain. In the first of these, in addition to a dense granular deposition in the inner layer of the roof, there are fairly numerous intracellular granules scattered through the whole depth

of the lateral walls; some of these granules adhere to the nuclear membrane, but many are distributed at random in the cytoplasm. A further phenomenon occurring in the diencephalon is the presence of ascorbic acid in the developing axons of some of the cells (Text-fig. 6); at this stage the cells concerned are confined to the ventrolateral region of the mantle layer. The pineal organ, which is represented by a small diverticulum, also contains ascorbic acid; the heaviest deposit is in the ependymal layer of the roof. Impregnation of



Text-fig. 5. Somitic cells of 4-day embryo.

developing axons can be observed in the midbrain and in the lateral walls of the spinal cord; in the latter this phenomenon is relatively inconspicuous. In the midbrain ascorbic acid also occurs dispersed in the body of the nerve cells. This is the case, too, in the ventrolateral part of the wall of the diencephalon, where the cells contain the highest concentration to be seen in the nerve-cells of the 4-day embryo.

The rudiments of the organs of the alimentary canal have at this stage, for the most part, little ascorbic acid. However, the liver and pancreas diverticula show a relatively heavy granular deposit, although histologically no difference

appears between their cells and the cells of the gut wall from which they are derived.

The distribution of ascorbic acid in the somites is indicated in Text-fig. 5. The dermatome cells contain almost none. In the myotome ascorbic acid is present in relatively large amounts, and can be seen outside the cells, attached to their processes, as well as in their cytoplasm; this is in accordance with the observation of Tonutti (1939) on developing muscle fibres, and is of interest since adult muscle is known to contain very little ascorbic acid indeed (Giroud, 1938, p. 69); moreover we have found that at a slightly later stage embryonic muscle contains no ascorbic acid which can be detected histochemically. The sclerotome at this stage contains less than the myotome; this confirms the preliminary report published by van Weel (1939).

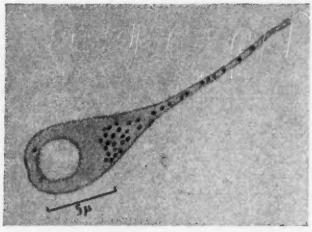


Fig. 6.

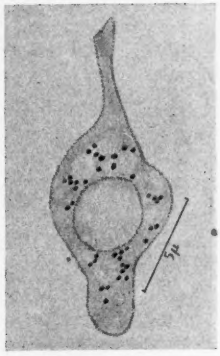


Fig. 7.

Text-fig. 6. Neurone from spinal cord of 4-day embryo, with ascorbic acid in axon.

Text-fig. 7. Cell containing fat (?) from mesenchymal tissue of 4-day embryo. Note ascorbic acid granules. See text.

In the neighbourhood of the dorsal root ganglia, and especially ventral to them, there are small groups of cells, distinct from those of the sclerotome and containing more ascorbic acid (Pl. 1, fig. 10). These cells seem to be the neural crest derivatives described by His (1892) as migrating ventrally in order to take part in the formation of the definitive sympathetic chain.

Two further types of cell remain to be described. Near the neural tube, in the undifferentiated mesenchyme which surrounds it, there are a very few, irregularly scattered cells which are quite distinct in appearance from their neighbours: they are highly refractile, owing to the presence in their cytoplasm of granules which have the appearance in the unstained preparations of fat droplets (Text-fig. 7). These cells cannot be distinguished in sections which have been stained with haematoxylin and eosin. Their interest lies in the fact that they contain ascorbic acid in association with the refractile material. In

addition to these very distinctive cells there are occasional cells indistinguishable from those of undifferentiated mesenchyme, except for the presence of ascorbic acid in granules attached to the surface of the nucleus.

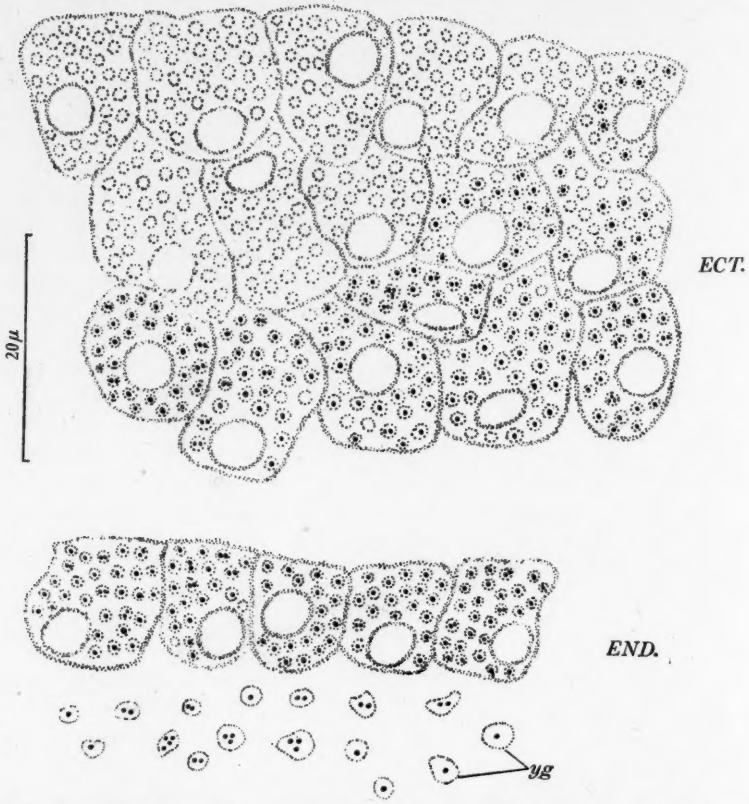
4. DISCUSSION

The association observed, of ascorbic acid with yolk granules, raises the question of its synthesis. There is very little information available regarding the mechanism of the synthesis of ascorbic acid in vivo. There is evidence that, as might be expected from its molecular structure, it is synthesized in plants from hexose precursors: Sah (1933) has put forward a theory of its formation from them in plant tissues. Guha & Ghosh (1933*a*, *b*) have brought evidence that mannose is a precursor of ascorbic acid in both animals and plants. The experiments of these authors with animal tissues have been repeated with negative results by Laporta & Rinaldi (1935), Scheunert & Schieblich (1937) and by Hawthorne & Harrison (1937). It therefore cannot be said that there is satisfactory evidence for synthesis from carbohydrates in animals.

Musulin *et al.* (1938, 1939) found that unsaponifiable fractions of halibut liver oil, oat oil and other vegetable oils, administered to rats, caused a marked increase in the rate of synthesis of ascorbic acid; these authors therefore suggested a lipoid precursor for the vitamin. Yolk granules contain a high proportion of lipoids, and the association observed between them and ascorbic acid in early embryos might be regarded as pointing towards synthesis from lipoids. The fact that the special cells, described above as apparently containing a large reserve of fat, also have ascorbic acid in association with it, is at least consistent with this suggestion. It may be added that the intracellular distribution of ascorbic acid observed in the extra-embryonic tissues does not conflict with this view: it is well established that ascorbic acid is associated with the "chondriome" in all cells which contain a high concentration of it (Bourne, 1935*b*; Giroud, 1938, p. 27); and though the mitochondrial system doubtless varies greatly in different types of cell, it has been shown to contain fats (Bensley & Hoerr, 1934). However, Musulin and co-workers, in continuing their investigations, have found that a wide variety of substances, including terpene derivatives and a number of aliphatic compounds, have an effect similar to that of fractions of animal and plant oils. These further results suggest that the substances used operate not as precursors, but by the indirect stimulation of the synthesis of ascorbic acid (Longnecker *et al.* 1939).

Although nothing can be concluded regarding the chemistry of the production of ascorbic acid, it is clear that the organs engaged in synthesis up to the end of the third day are the extra-embryonic tissues and the yolk granules below the blastoderm. Glycogen is already known to be laid down extra-embryonically until the liver becomes functional. The concept of an extra-embryonic "transitory liver", based on the latter fact, is thus strengthened by the observed distribution of ascorbic acid (see Needham, 1934).

The absence of ascorbic acid from the embryonic tissues during the first 3 days, and its sudden appearance in several tissues during the fourth, raise the question of the precise role of ascorbic acid in early development. There is little in any of the previous work on the physiology of vitamin C to provide guidance on this question. There is only one group of developmental processes in animals



Text-fig. 8. Semi-diagrammatic representation of part of the unincubated blastoderm photographed in Pl. 1, fig. 9, showing intra- and extracellular yolk globules (*yg*), some of them showing the presence of ascorbic acid.

in which it is certain that ascorbic acid plays an essential part: these are the laying down of the intercellular materials of connective tissue and bone (see Dalldorf, 1939, p. 339). There is extensive evidence, summarized by Giroud (1938, pp. 152 *et seq.*), that in flowering plants the presence of ascorbic acid is essential for the rapid growth of embryonic tissues, but no comparable observations have been carried out on animal material. The authors have found that chick heart fibroblasts and osteogenetic cells give little or no reaction

when growing rapidly in a medium containing an optimal amount of embryo extract (Willmer & Jacoby, 1936). Adrenal cortical and medullary cells from chicks of 17 days' incubation were also cultured in these conditions, with the same results. It is therefore clear that the presence of quantities of ascorbic acid in the reduced state is not necessary for the rapid growth of animal tissues.

It might be supposed, on the basis of the observations described above, that ascorbic acid plays no part in the development of the chick until the special processes of histodifferentiation have begun, but this does not account for its presence in high concentration in the extra-embryonic tissues. Perhaps the most likely explanation of the facts is that ascorbic acid is utilized by the early embryonic tissues, but that the oxidation potential which prevails in them is so high that ascorbic acid is oxidized immediately on reaching them. In the preparations of embryos of from 1 to 3 days there is usually a very faint black precipitate on the inner walls of the blood vessels, and of the lumina of the coelomic cavities and the neural tube; this may be due to the passage of ascorbic acid in the plasma and tissue fluids, from the extra-embryonic tissues into the embryo.

5. SUMMARY

1. A study has been made of the distribution of ascorbic acid (vitamin C) in the cells and tissues of chick embryos, from the unincubated blastoderm to the end of the fourth day of incubation.

2. The acid silver nitrate method was used for the demonstration of ascorbic acid. The validity of this method is discussed.

3. In unincubated blastoderms, and in those of 12 and 18 hours' incubation, evidence of ascorbic acid was found in association with yolk granules both within and outside the cells. The cells seen to contain ascorbic acid were confined to the periphery of the blastoderm.

4. In embryos of from 1 to 3 days the acid silver nitrate reaction shows the presence of ascorbic acid only in the extra-embryonic tissues, in particular in the ectoderm and endoderm. The cells of these layers contain large quantities of it dispersed in granules throughout the cytoplasm.

5. During the fourth day the silver nitrate reaction indicates the presence of ascorbic acid in the central nervous system, the liver and pancreas diverticula, and some mesoderm derivatives including the myotome. Its distribution at this age is described in detail.

6. The results are discussed with reference to the mechanism of synthesis of ascorbic acid in vivo, and to its role in development.

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EXPLANATION OF PLATE 1

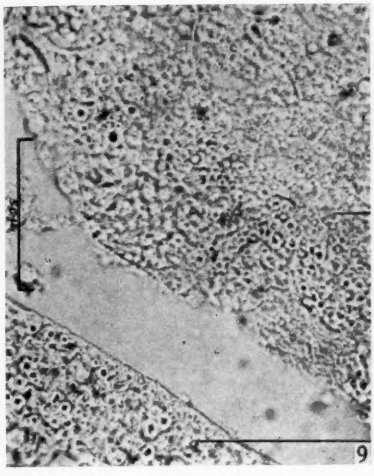
Photographs of unstained sections cut at 5μ

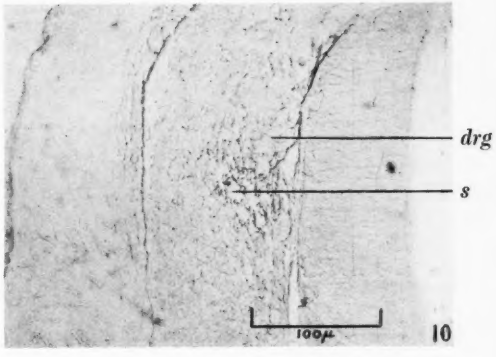
Fig. 9. (See also Text-fig. 8.) Unincubated blastoderm showing yolk globules with black precipitate; *ep* = epiblast, *en* = endoderm. Transverse section.

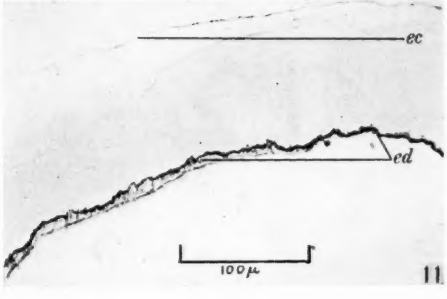
Fig. 10. Transverse section of 4-day embryo showing dorsal root ganglion (*drg*) and sympathetic cells containing ascorbic acid (*s*).

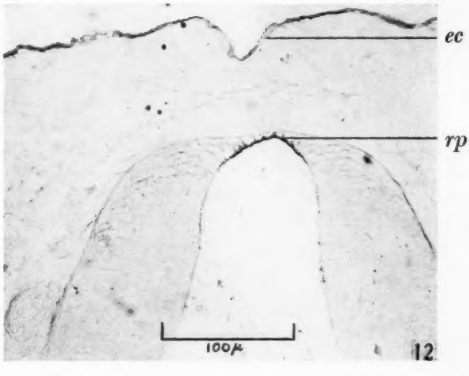
Fig. 11. Transverse section of 4-day embryo showing impregnation of dorsal ependymal layer of diencephalon (*ed*); *ec* = ectoderm.

Fig. 12. Transverse section of 4-day embryo showing impregnation of roof-plate of spinal cord; *rp* = roof-plate, *ec* = ectoderm.









BARNETT AND BOURNE—ASCORBIC ACID IN THE EARLY EMBRYO

UNIV. OF MICH.

ANATOMICAL NOTES

A STEREOSCOPIC X-RAY METHOD OF RECONSTRUCTION

By W. R. M. MORTON
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The ordinary wax-reconstruction methods show the contours of a solid or hollow organ fairly clearly, but it is difficult to make such a reconstruction of a solid, or solids, embedded in a solid matrix. One way of doing this is to represent the matrix as a space, but if the embedded solids are small and numerous, the technical difficulties of holding them in position, without obscuring the main details of the model, are considerable. The alternative method of reconstruction by drawing on glass plates is often useful, but if the model is narrow and of fairly uniform diameter in its short axis, there is difficulty in getting a clear view in a plane some distance from the surface of the glass.

The problem of showing the grouping of anterior horn cells in the spinal cord of foetal lambs led to the development of a radiological method of reconstruction. The first attempt was by means of lead shot embedded in wax plates, each shot representing one or more cells. A normal wax reconstruction was made of the spinal cord of a foetal sheep, and shot embedded in each plate in groups to correspond with the grouping of the cells as depicted on the enlarged drawing. Stereo X-ray photographs were then taken of the reconstruction. On examination of the photographs it was found that the stereoscopic effect was very good except where several pellets of lead overlapped each other. More than two pellets gave the same density of shadow on the negative. In order to get a wide range of density, red lead and starch paste were tried as a substitute for the shot, and finally a lead paint (Newton's poster colour, chrome lemon) was used. Cardboard sheets were used instead of wax plates. The method used was as follows:

Rectangular cardboard strips $12 \times 3\frac{1}{2}$ in. were taken, co-ordinates were ruled 6 and 3 in. from the bottom and left side respectively. The image of the section was projected on the cardboard by the Edinger enlarging apparatus at a suitable magnification, and orientated in such a manner that the midline of the cord lay along the y-axis (coronal sections of spinal cord) and the centre of an easily identifiable dorsal root ganglion along the x-axis. A pencil outline of the cord was then made and the cells represented by painting directly on to the cardboard with lead paint, using a fine brush. It was difficult to represent each individual cell at magnifications below 50, as the minimal effective spot of paint had a diameter of about 1·5 mm. However, it was quite possible to indicate cell groups at a magnification of 25. The resulting series of cardboard strips were then superimposed on each other, the correct intervals between each layer being obtained by inserting paper or cardboard strips to the required thickness. Two holes were bored in the free margin and the strips were bound together with wire and gummed paper. A firm rectangular block was thus formed which could be easily handled.

Stereoscopic X-ray pictures were then taken of this block on ordinary Ilford films, the tube being 30 in. from the plate. In some instances a normal shift of $1\frac{1}{2}$ in. in either direction was used, but when it was desired to increase the stereoscopic effect for any reason, a double shift, i.e. 3 in., in either direction was used. The model was placed flat on the table, or at any desired angle previously determined by screening. The model was stationary and the film changed with each shift of the tube. A fine focus tube was used, the exposure and penetration varying with the thickness of the model.

A slightly different procedure was used to show fibre tracts in the brain of foetal sheep, the tracts reconstructed being the descending root of the trigeminal nerve and the tractus solitarius. The enlarged images were drawn on paper sheets so that the midline of the brain came on a previously drawn y-axis line. The fibre tracts were drawn in, and later painted in, using the microscope as a guide to the final form of the paint lines. When dry, the papers were superimposed on each other over an illuminated glass sheet, firmly clamped together between two pieces of wood, and trimmed off at the bottom edge. Two fine holes were then drilled through the paper and wood; cardboard of suitable size and thickness was then put in the clamp and drilled through the same holes in the wood. The cardboard and paper were interleaved and held together by fine brass nails running through the drill holes.

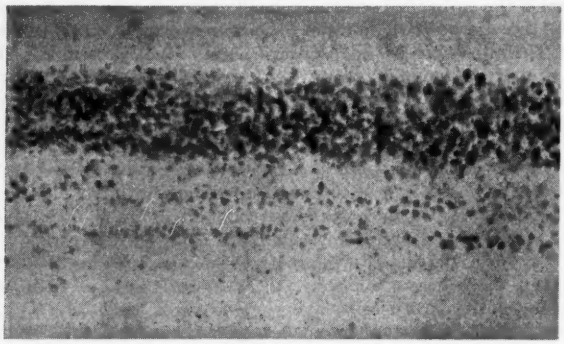

Fig. 1. X-ray photograph of reconstruction of anterior horn cell columns of cervical spinal cord in a 67-day sheep foetus. ×22.

The resulting stereoscopic pictures showed quite clearly the various cell columns and fibre tracts, and gave a very good idea of their spatial relationships (see figure). It is thought that the method may be of use in teaching students the internal anatomy of the central nervous system, as well as being of use to the research worker.

I am indebted to Prof. H. A. Harris for useful suggestions regarding radio-opaque media and for permission to use the radiology department, and to Mr J. A. F. Fozzard for carrying out the radiological work.

NOTE ON THE HISTOLOGY OF LYMPH NODES IN *ECHIDNA*

By M. A. BASIR
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The following account of the structure of the mesenteric lymph nodes in *Echidna* is intended to fill a conspicuous gap in the literature dealing with monotreme anatomy.

Mesenteric lymph nodes of *Echidna* were available from a specimen which had been fixed in formalin and preserved in alcohol for a number of years. Serial sections of these nodes were cut after embedding them in paraffin by the usual method, and were stained with Ehrlich's haematoxylin and counterstained with eosin, Pasini, Dominici and Mallory's connective tissue stain. In order to supplement the observations on the *Echidna* lymph nodes, an examination was also made of the lymph nodes of the platypus, rabbit, rat, and guinea-pig. These were fixed in formol, Zenker, and Bouin, and stained by the same methods.

The supporting framework. The capsule (*c*) of the lymph node of the *Echidna* is really an expansion of the wall of the lymph vessel and consists mainly of collagenous tissue (*c.t.*). It also contains a large number of smaller lymph channels (*l.c.*). Lying beneath the capsule there is a large subcapsular lymph sinus (*c.l.s.*) which separates it from the surface of the node. Thus the whole node appears to lie bathed in lymph.

It is evident, however, from the diagram that the capsule is attached to the node in two places (h_1, h_2), where the capsule is somewhat thickened. At h_1, which corresponds to the hilum of the lymph node, there are seen (cut in section) the blood vessels and afferent lymph channels. At the opposite pole of the lymph node (h_2) the capsule contains a few blood vessels and a large number of efferent lymph channels.

Unlike the rat, rabbit, and guinea-pig, there is a striking absence of trabeculae extending into the substance of the lymph node, both in *Echidna* and *Ornithorhynchus*. The supporting framework of the lymph node therefore consists mainly of a network of reticular fibres and a few connective tissue fibres. These reticular fibres are firmly attached to the capsule of the node. The network of the reticular fibres is dense and narrow at the periphery and somewhat loose and more open towards the centre of the lymph node. Pale-staining, polygonal, reticular cells lie close alongside the reticular fibres.

The structure of the lymph node. The mesenteric lymph node of *Echidna* is essentially a single lymph follicle which can be divided into an outer cortical and an inner medullary part. Microscopically the demarcation between the cortex and the medulla is relatively sharp and the cortex is completely surrounded by the medulla. But the lymphatic cords characteristic of the medulla of the lymph nodes in most mammals are not apparent here. The structure of the medulla resembles very much the germinating centre of Fleming found in the mammalian lymph nodes. There is a preponderance of the medullary substance over the cortical as is usual with the nodes of the peritoneal cavity. The lymph channels here have no walls of their own and so appear as mere spaces in between the reticular fibres.

The cortex appears as a uniform mass of small lymphocytes (*s.lym.*) closely embedded in the narrow meshes of the reticular fibres. It lies in immediate contact with the subcapsular sinus (*c.l.s.*); it may therefore be presumed that lymph from this sinus percolates through the lymphoid tissue of the node.

The inner medullary portion (*g.c.*) consists of large lymphocytes loosely embedded in an open meshwork of reticular fibres. These lymphocytes have a lightly staining cytoplasm and a slightly excentric oval nucleus.

The blood supply of the node is mediated by blood vessels at the hilum and also by a few vessels which enter directly from the capsule. Capillary vessels are seen in greater number in the cortical region than in the medullary substance. Because of the absence of a trabecular system and medullary cords in the lymph nodes of the monotremes, the blood supply to the medullary substance is poor as compared with that of the rabbit and rat.

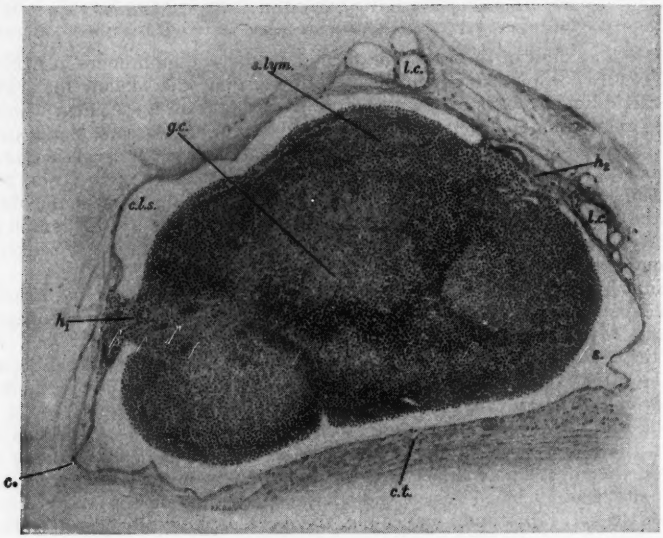

Fig. 1. Section of the lymph node of *Echidna*. Stained by haematoxylin and eosin. ×50. *c.* capsule; *c.t.* connective tissue; *g.c.* germinating centre; h_1, h_2, hilum; *l.c.* lymph channel; *c.l.s.* subcapsular lymph space; *s.lym.* small lymphocytes of the cortex.

I wish to express my thanks to Prof. J. P. Hill, F.R.S., for the supply of the material, and to Dr D. Krauz for the drawing which appears in the accompanying figure.

A HUMAN OVUM AT THE PREVILLOUS STAGE

By J. H. DIBLE and C. M. WEST

The Department of Pathology, British Postgraduate Medical School, London, and the Department of Anatomy, University College, Cardiff

The specimen which is described in this communication consists of what is believed to be a normal ovum that has been not long imbedded in the uterus, and it represents the youngest stage of human development that has so far been recorded in this country. It was discovered by one of us (J. H. D.) during the course of a post-mortem examination of a woman, aged 28, who had died from internal hydrocephalus due to fibrous adhesions at the base of the brain which were probably the result of trauma. The post-mortem examination was made not more than ten hours after death, and there is no reason to suppose that the specimen is not normal.

A large corpus luteum was seen in one ovary and this directed particular attention to the uterus; on opening this along the anterior wall the mucous membrane was found to be thick and velvety, and on the posterior wall, towards the fundus, there was seen a small vesicle (Fig. 1[1]) projecting above the surface which was thought to be an early ovum. A small wisp of fibrinous material was adherent to the summit of the vesicle. No menstrual history was available.

The opened uterus was fixed in formalin and, after being photographed, a block of tissue containing the vesicle was removed, cut at 4μ, and stained with iron-haematoxylin and eosin; blocks of tissue from other parts of the uterus were also removed for study of the structure of the endometrium at a distance from the vesicle; this endometrium presents the characters typical of the premenstrual phase, with large, tortuous and active glands, and a rich blood supply; the sections show, too, that the fixation and preservation are good. In the sections of the block of tissue containing the vesicle the mucous membrane is 5 mm. thick and is divisible into a stratum compactum, 0·3 mm. thick, and a stratum spongiosum (Fig. 2).

The implantation site can be recognized in the sections with the unaided eye by the pink stain which has been taken up by some dilated glands lying at the base of the area. Contrary to what has been found in several young specimens, and to what might have been expected from the appearance of the intact vesicle, the implantation site is not much raised above the surface in the sections. Implantation has occurred between the mouths of two glands which are bent round the ovum, and the site of implantation occupies the stratum compactum only. The ovum is not covered with a decidua capsularis,

[1] Figs. 1–11 are shown on Plates 1–6.

and the point of entry of the ovum into the endometrium is seen (Figs. 5, 7) as a crater-shaped area, from which the uterine epithelium is deficient, overlying the summit of the ovum. The aperture of entry is 0·23 mm. wide; it is filled, as with a bung, by the 'operculum' (Teacher, 1924) and is capped by the 'closing coagulum' or 'Gewebspilz' (Peters, 1899). The latter appears as a loose mass of fibrinoid material containing some leucocytes and it was seen as the small wisp, referred to above, when the uterus was opened.

The ovum (Fig. 3) consists of a slightly flattened chorionic vesicle, the blastocyst, which has an internal measurement of 0·47 mm. in the equatorial plane, i.e. from side to side, and of 0·28 mm. in the polar plane, i.e. from the surface inward. The inner surface of the blastocyst is smooth and has the embryonic rudiment attached at its base; the outer surface is irregular owing to cellular and syncytial processes growing out from it into the implantation cavity. These processes are stained deeply with haematoxylin and they stand out in sharp contrast with the clear cavity in which they lie (Fig. 4). The smooth inner surface of the blastocyst is lined with primary mesoderm, and inside this there is the exocoelomic vesicle (Heuser, 1938) enclosed by the exocoelomic membrane (Heuser's membrane). The embryonic rudiment is composed of a thick ectodermal plate which forms the ventral wall of the amniotic cavity, and of a rather thinner entodermal plate which is continuous at its margins with the exocoelomic membrane. There is no yolk sac.

There are no chorionic villi and thus the specimen belongs to the group of ova at the previllous stage, which includes the Miller (1913, and Streeter, 1926), the Kleinhans (Grosser, 1922), the Müller (1930), and the two new Hertig ova of which a preliminary account only has so far appeared (Hertig & Rock, 1939). Further, it corresponds with Streeter & Wislocki's (1938) stage II for the macaque, which they term the 'stage of trophoblastic lacunae' and which they estimate to belong to the period from the 11th to the 14th day. The implantation cavity appears (Figs. 4–6) as an almost empty, clear and circumscribed area and thus stands out in sharp contrast with the adjacent endometrium on the one hand and the darkly stained trophoblast on the other; it is this character of the implantation cavity which gave to the specimen a cystic appearance when first seen in the uterus with the unaided eye.

The trophoblast. Growing from the outer surface of the blastocyst are the trophoblastic processes. In the account which follows, the terminology employed by Ramsey (1938) in her description of the Yale embryo will be used. Thus we are able to recognize central cytotrophoblast, peripheral cytotrophoblast, and plasmoditrophoblast or syncytium.

The central cytotrophoblast (Figs. 7, 8) forms the true wall of the blastocyst; it is composed of a single layer of lining cells which, for the most part, appear rather condensed and flattened and have darkly stained, round or oval nuclei, but poorly defined cell walls; in some sections the nuclei are packed closely together giving a dark border to the blastocyst, while in others, particularly in the region of the operculum, they are few and far between.

The peripheral cytotrophoblast (Figs. 4–8) appears as sheets, or columns, of cells growing from the blastocyst wall into the implantation cavity; in other words, it is an extension outward of the central cytotrophoblast. The cells are larger and have more clearly defined walls than have those of the central cytotrophoblast, their nuclei generally are not so darkly stained, nor do they fill the cell to the same extent, so that there is often a clear space left between the nucleus and the cell wall. Beyond the peripheral cytotrophoblast, and also extending in amongst its cell columns, are grey, filmy masses and strands which may be nucleated or non-nucleated; this tissue is the plasmoditrophoblast, or syncytium (Figs. 5–8). It is devoid of any cell boundaries, and in some places nuclei occur in clumps, like the 'heaped-up epithelial nuclei' described by Streeter & Wislocki (1938).

In the plasmoditrophoblast brown granules are seen in many places; similar granules have been described by Johnstone (1914) and Greenhill (1927). Johnstone suggested (p. 270) that they are composed of molecules of blood pigment adherent to a minute droplet of fat, the fat itself having disappeared in the course of the process of fixation and staining. We are of opinion that this pigment is a formalin-haemoglobin precipitate, and the fact that it is found mostly at the implantation site and in the cells of distended glands which form lacunae of blood rather supports this view, since these are sites of haemorrhage, and it is well known that in slightly autolysed tissues such precipitates are common in histological material. The ovum, and the autolytic ferments present in the trophoblast, may well be responsible for some degree of local autolysis. Greenhill implies (p. 340) a similar origin of the granules in his specimen. It has not been possible to make any microchemical analysis of these granules as it would have involved an interruption in the series of slides, but it may be mentioned that they are anisotropic, as are the formalin precipitates. The frequent occurrence of these granules in association with leucocytes provides further support for their origin from blood.

Several authors have referred to the foam-like condition of the syncytium of early stages due to the presence of innumerable vacuoles of various sizes, and the general opinion seems to be that this vacuolated syncytium has been derived from a more solid plasmodium which has grown out from the blastocyst wall. Thus Bryce (1908) suggests (p. 43) that the spun-out condition of the plasmodium or syncytium is due to the formation in the vacuoles of a digestive fluid which, after rupture of the vacuoles, is replaced by maternal blood; the large amount of blood in the implantation cavity is a striking feature of the ovum T.B. 1. Streeter (1926), in his account of the Miller ovum, describes the lacunae as being small near the wall of the ovum and larger as the periphery is approached, and he refers to them (p. 37) as representing the 'histological picture of a process by which a solid mass of trophoblast becomes converted into a sponge-like syncytium'. The Miller ovum shows a less definite implantation cavity than the present specimen.

In our ovum the vacuolated network (Figs. 7, 8) seems to be made up

largely of coagulated protein with branching cells scattered through it; these cells appear to be partly trophoblastic and partly stromal, and some are degenerate. This appearance is no doubt produced, so far as the protein is concerned, by fixation, and it seems probable that in life the contents were mainly fluid or gelatinous with strands of fibrin, amongst which the cells of the trophoblast were growing out from the blastocyst as in a tissue culture. We believe that our ovum, the Miller ovum, and the ovum T.B. 1, show progressive stages in the dissolution of the originally more solid plasmodium, the present specimen being at a stage intermediate between the Miller ovum, and the ovum T.B. 1, where the vacuoles have become filled with blood. The Müller ovum we believe to be at a stage similar to ours. The embryo Kleinhans, judged by the condition of the implantation cavity, seems to be at a stage intermediate between the present specimen and the ovum T.B. 1, for it shows a cavity which is fairly clear cut but which contains rather more blood than is present in our specimen. By the same criteria, the two new Hertig ova are both at stages earlier than either the present specimen or the Miller ovum; Hertig's no. 7700 in particular shows an almost solid plasmodium surrounding the trophoblast wall.

Teacher (1924) refers to the two generations of syncytium, the destructive and temporary syncytium—the 'Implantations-syncytium' of Grosser—and the permanent syncytium of the chorionic villi—the 'Zotten-syncytium' of Grosser. It is this first generation of syncytium that we have before us, and we get the impression that it shows evidence of destructive action and also of being in a process of degeneration, for it is a degenerate tissue and yet it seems able to open into capillaries and, contrary to what Streeter found in the Miller ovum, into glands also. Streeter further stated that he was unable to find any evidence of destruction or ingestion of the stroma cells by the syncytial loops; in the present specimen, as serial sections are examined from the non-implantation area towards the blastocyst, the first change seen is that the stroma becomes oedematous, the spaces between the cells are increased, and their nuclei become enlarged and paler. By the appearance of their nuclei these maternal stroma cells can be distinguished from the cells of the trophoblast, and in sections through the periphery of the implantation cavity they can be recognized without any doubt, though they are not numerous and their period of survival would appear to be short; here and there they can be seen lying in a mass of the filmy grey tissue that has been recognized as syncytium.

It may be suggested here that much of the lacy, network-like, appearance of the trophoblast seems to be due to destruction of its cells, by autolysis or by extracellular digestion, or by a combination of both processes; the general picture suggests the presence of some rather active ferment, or of conditions causing autolysis. We believe that many of the spaces between the trophoblastic branches may contain autolysed blood, the more so because the red cells in many of the capillaries in the vicinity are autolysed, though not in those farther away. This interpretation is in conformity with the suggestion we made above concerning the origin of the pigmented granules. Thus, there does seem

to be evidence of the digestion of the stroma cells by the syncytium in this specimen, and we believe that such destruction must have been extensive, for there is little evidence of the stroma having been simply pressed outward, as Streeter found to be the case in the Miller ovum, though in some places it is arranged more or less in concentric lamellae as if it had been pushed aside to some extent. It does seem that the main method of enlargement of the cavity, with the necessary removal of the stroma cells, is by a process of oedema of the stroma, degeneration of its cells and their autolysis or digestion. It is noteworthy that there is a strip—the 'border-zone'—of degenerated and necrotic stroma all round the implantation cavity, except where it is in contact with a gland (Figs. 4, 5).

The implantation cavity. The implantation cavity measures 1·0 mm. in its equatorial diameter and 0·575 mm. in its polar. As has been mentioned above, it appears as an almost empty, clear and circumscribed area, and though this character of emptiness is an appearance only, yet in this character and in the sharp outline of the cavity the specimen shows a condition different from what is found in other early ova, with the exception of that described by Müller, in that these have an implantation cavity more or less filled with blood, and with an irregular and indefinite outline. The cavity rests on two glands which have been so greatly distended with blood that their epithelium has been quite flattened and resembles the endothelial lining of a blood vessel, for which it was at first mistaken (Figs. 3, 5 and 8). The distension affects mainly the deeper parts of the glands. Brewer (1937) states that in all human pregnancies reported there is an accumulation of blood in the uterine glands, and, in the Edwards-Jones-Brewer ovum which he describes, the glands are certainly very distended with blood, but the only specimen besides the present one that shows a comparable distension is that described by Herzog (1909). As to the cause of the distension in the present specimen, it is clear that in the case of one gland at least it is due to the fact that it does not open on the surface, its lumen being occluded with syncytium and its mouth incorporated in the implantation cavity. It is therefore distended in part with blood, and in part with its own secretion which has no exit. In another case, the gland has burst on to the surface (Fig. 6) and its contents have been expelled into the uterine cavity where they form a wisp of fibrinous material impregnated with leucocytes.

The closing coagulum and the operculum. A wisp of fibrinous material has been alluded to already as the closing coagulum which caps the aperture of entry of the ovum into the uterine mucous membrane (Figs. 1, 3, 5, 7 and 8). The structure of this coagulum and of the contents of the implantation cavity is essentially similar, and it may be regarded as a part of the contents of the cavity which has become free on the surface owing to the absence of any decidua capsularis, and to the disappearance of the uterine epithelium over the point of entry of the ovum. The closing coagulum is also continuous in part with the contents of the gland which has burst on one side of the ovum. The

very thin covering of the ovum was recognizable in the intact specimen, for the ovum had the appearance of a tiny cyst, like a blister in the thick mucous membrane, with its pole glistening through the thin covering. It is interesting to note, in connexion with the origin of the closing coagulum, that Brewer (1937) showed a much larger and more organized 'blood clot' overlying the surface epithelium at the implantation site, and he suggested that its character indicated previous bleeding into the uterine lumen; the implantation cavity in Brewer's ovum contains a good deal of blood, and this may explain the difference in the appearance of the closing coagulum in his specimen and in ours.

The implantation cavity does not surround the ovum completely, for in its superficial part it is interrupted by the presence of the operculum (Figs. 2, 5). As so well described by Schlagenhaufer & Verocay (1916), quoted by Teacher (1924, p. 179), the operculum, 'as the stopper of a bottle, as the keystone of a vault...closes the entrance to the implantation cavity with its precious contents'. It is clearly an outgrowth of the central cytotrophoblast at the superficial pole of the ovum, and it fills the space between this part of the ovum and the uterine cavity. In structure it is like the peripheral cytotrophoblast of the rest of the ovum, except that it is rather less cellular and more vacuolated; in it are some small masses of syncytium and a few degenerated maternal stroma cells. The whole mass has a degenerated appearance and gives the impression of being a tissue, derived from proliferation and outgrowth of the cytotrophoblast, which has nearly outlived its usefulness. In some sections (Fig. 8) the operculum and the closing coagulum are continuous. Over the aperture of entry the uterine epithelium is deficient (Figs. 5, 7). Farther laterally it forms the sides of the crater-like area occupied by the operculum, but both here and in the adjacent glands it is flattened and has the appearance of having been stretched. This is quite unlike the appearance of the epithelium lining the uterus and the glands generally, which is columnar.

The blood vessels. Fig. 9 is a photograph of a section through the middle of the ovum, and on it have been marked with ink all the blood vessels. It shows that the endometrium is well supplied with blood. Small, thick-walled, spiral arteries and larger and thinner veins are seen in the stratum spongiosum, but capillaries only are present in the stratum compactum. There is no definitive blood sinus at the base of the ovum, such as is described by von Möllendorff (1921*a*) in the embryo 'Sch.', and by Ramsey (1937, 1938) in the Yale and Lockyer embryos; instead, there are numerous small vessels round the implantation site which ultimately join into two main veins, one rather larger than the other. The capillaries of the stratum compactum are opened by the syncytium and become incorporated in the implantation cavity, giving rise, at least in part, to the clear spaces of the cavity.

The embryonic rudiment. The embryo is found on the inner surface of the basal wall of the blastocyst. It consists of a thick ectodermal embryonic plate which forms the ventral wall of an otherwise thin-walled amniotic cavity, and

of a rather thinner entodermal plate which has not yet differentiated into any part of a definitive yolk sac. Spreading out from the sides of the amniotic cavity are the cells of the primary mesoderm which, when traced in a lateral direction, form a continuous lining for the inside of the blastocyst (Figs. 7, 8, 10 and 11). Within this primary mesoderm there is an even thinner layer (Figs. 5, 7 and 8), the exocoelomic membrane (Heuser, 1938); this encloses a large, more or less spherical, cavity which we thought at first was the yolk sac, admittedly a good deal larger than we had expected to find in an ovum at this stage. This membrane will be referred to in more detail after the other parts of the embryo have been described. It is unfortunate that a number of sections containing the embryo have been lost and it is thus not possible to make a complete reconstruction, but the following are the measurements of the embryo taken in the middle of the ten sections in which it occurs:

Embryonic disc	0·1 × 0·02 mm.
Endodermal plate	0·14 × 0·016 mm.
Amniotic cavity	0·04 × 0·09 mm.

The cells of the ectodermal and entodermal plates do not differ much; in each (Figs. 10, 11) the cells are closely packed, here and there piled up two deep, but standing in a columnar arrangement in the ectoderm and lying end to end in the entoderm. The cell boundaries are not well defined, but the nuclei are large and granular, exceeding those of the central cytotrophoblast but equalled by some of those of the peripheral cytotrophoblast.

In a discussion of the formation of the human yolk sac Stieve (1931) shows a section through the embryo Werner which resembles our embryo in having what he thought to be a large yolk sac. This was our interpretation also at first, but we have since had the opportunity and advantage of seeing Dr Streeter's beautiful photographs of the two new Hertig ova, of having his personal opinion about them, and of having read Heuser's (1938) paper on the 'Early development of the primitive mesoblast in embryos of the rhesus monkey'. The stages illustrated in Heuser's publication are of particular interest, for Streeter (1933) has shown that the difference in detail of form and in rate of differentiation and growth between man and monkey does not become appreciable till the end of the second month, and that known ages of macaques may be transferred to human embryos at least for the first month or six weeks. It is therefore profitable to compare our ovum with the stages of the macaque described by Heuser, since, as we have suggested above, our specimen falls into the stage of development which Streeter & Wislocki attribute in the macaque to the period from the 11th to the 14th day, and Heuser gives photographs of embryos of the 11th, 12th and 13th day.

The most striking difference between each of these specimens and our ovum is seen in the condition of the entoderm. We have described the entoderm plate as being nearly equal in thickness to the ectoderm plate and have stated that there is no definitive yolk sac. Heuser's 11th day embryo shows no yolk sac, but in the specimen of the 12th day it is just beginning, and in the specimen

of the 13th day it is seen clearly. In respect of the development of the yolk sac, then, our embryo corresponds with one of the 11th day in the macaque, the chief difference being in the thickness of the gut entoderm which, in the macaque, consists of a flattened layer of only one cell in thickness spread out on the inner surface of the inner cell mass.

Heuser states (p. 384) that 'Cells delaminating from the inner surface of the thickened trophoblast seem to be continuous with the primitive entoderm beneath the inner cell mass'. He finds that by the 11th day these cells become continuous with a layer that has split off from the ventral surface of the entoderm sheet so that a complete envelope, the exocoelomic membrane, is formed, having an origin partly trophoblastic and partly entodermal. In our ovum such a membrane is present, but its origin is not as clear as in the macaque; it appears (Figs. 3, 8, 10 and 11) to be continuous in the embryonic region not with a layer of cells split off from the entoderm but with the edges of the entoderm itself, nor is it certain (though it is probable) that the remainder has arisen by a splitting off from the inner surface of the trophoblast. It thus appears that, as far as the development of the exocoelomic membrane is concerned, our ovum is at a stage earlier than that of the macaque at the 11th day.

We have referred above to the primary mesoderm as forming a continuous lining for the inside of the blastocyst. Heuser finds that till the 13th day, 'except in a small zone around the embryo itself, there is little if any additional mesoblastic tissue', that is, additional to the tissue that has formed the exocoelomic membrane. Our ovum has a trilaminar blastocyst (Fig. 7) composed of an outer trophoblast, a middle primary mesoderm, and an inner exocoelomic membrane; it thus corresponds, as regards the development of the mesoderm, with a macaque of some days later than the 13th.

The primary mesoderm looks as if it might well have been derived from a splitting off of cells from the inner surface of the trophoblast, and in the embryonic region it is continuous with cells lying at the sides of the ectodermal plate and the amnion (Figs. 8, 10 and 11). These cells extend for some distance outward from the embryo and help to fill the space between the trophoblast and the exocoelomic membrane. This is seen particularly well in a glass-plate reconstruction in which the mesoderm cells are shown to be more numerous here than elsewhere.

With regard to the entodermal plate, we are unable to see more than one form of cell, though, in some sections, on the ventral surface of the plate there are a few cells which are less darkly stained than the majority, and we can see no film of flattened cells which might give rise to the basal portion of the exocoelomic membrane; nor can we see any splitting off of cells from its ventral surface to form the ventral wall of the yolk sac. We believe our specimen to be at a stage antecedent to the completion of the exocoelomic vesicle.

For some years it has been believed that the human yolk sac appears early in development and is already present in the youngest known specimens, that

the yolk sac develops by a process of cavity formation in the ventral part of the inner cell mass, and that all its walls are of entodermal origin. It has been believed that these conditions hold good for other primates also. For example, J. P. Hill (1932), in his Croonian Lecture, described the condition of the yolk sac in the earliest catarrhine embryo then known, the 'Keim S' of *Nasalis larvatus* (Selenka); he believed it was at 'the stage when an originally solid endodermal yolk-sac primordium is in course of becoming converted into a hollow vesicle by a process of vacuolization' (p. 108).

More recent work on primate material by Heuser, Stieve, and Streeter has cast some doubt on the truth of these older theories, and Streeter (1937), in discussing the origin of the yolk sac in primates, states that its earliest cells are differentiated 'as a thin membrane between which and the gut endoderm there arises a narrow cleft. This cleft becomes rapidly distended and so forms the conjoint yolk sac cavity and gut cavity. This cavity is therefore dual in origin and is bordered on its dorsal part by cells that are to form the gut endoderm, an induced product or migratory element from the embryonic ectoderm. It is bordered on its ventral part by the yolk sac endoderm which is a derivative of the primitive mesoblast and is essentially trophoblastic.'

Through Dr Streeter's kindness we have had the pleasure of examining photographs of the two new Hertig ova and of an ovum of the macaque at about the same stage of development. The Hertig ova are at much the same stage as our own and, like it, show ectodermal and entodermal plates and an exocoelomic membrane which appears to be continuous with the edges of the entodermal plate. The macaque ovum is at a rather later stage, and in it the exocoelomic membrane is separate from the entodermal plate and forms a closed exocoelomic vesicle on the ventral side of the entoderm. A yolk sac is present, and consists of a small vesicle with its dorsal wall formed by the entodermal plate and its ventral wall by cells which look different from those at the base; they are smaller, more flattened, and have more darkly stained nuclei, and they resemble the cells of the primary mesoderm and the dorsal wall of the amniotic cavity.

The study of our own specimen and of the photographs of the Hertig ova and the macaque lead us to believe that the older theories must be revised; for we find that the Hertig ova and our own, the youngest human specimens thus far known, have no yolk sac. The macaque ovum strongly suggests that the yolk sac does not arise by a process of cavity formation in the inner cell mass, and shows that the cells of the dorsal wall of the yolk sac are quite different in appearance from those of its other walls. Streeter's (1937) suggestion, quoted above, at first filled us with astonishment and confusion, for it seemed that, in his view, there was no such thing as entoderm *sui generis* if the gut entoderm is a migratory element from the embryonic ectoderm, and if the yolk sac entoderm is derived from the primitive mesoblast and is trophoblastic in origin. The difficulty, however, seems to be one of terminology

and of the distance back in their developmental history to which the different layers are traced.

After careful study, we agree with what we believe to be Streeter's views and the course of development seems to us to be as follows: The inner cell mass becomes differentiated into a thicker dorsal part, the embryonic ectodermal plate or embryonic disc, and a thinner ventral part, the embryonic entoderm. The exocoelomic membrane splits off from the inner surface of the trophoblast and becomes adherent to the edges of the embryonic entoderm, so that there is formed a temporary exocoelomic vesicle bounded dorsally by entoderm, and elsewhere by cells split off from the trophoblast. A layer of cells splits off from the ventral surface of the embryonic entoderm and joins with the cells of the exocoelomic membrane, or else the exocoelomic membrane grows round on the ventral surface of the entoderm and then becomes separated from it. Thus there is formed finally a complete exocoelomic vesicle, detached from the entoderm, occupying the interior of the blastocyst. The primary mesoderm, which has meanwhile been split off from the inner surface of the trophoblast outside the exocoelomic membrane, spreads as a thin layer over the ventral surface of the embryonic entoderm, between it and the exocoelomic vesicle. A cleft then appears between this film of primary mesoderm and the embryonic entoderm, so that there is formed a cavity bounded dorsally by the cells of the embryonic entoderm, and elsewhere by cells derived from the primary mesoderm. This cavity is the yolk sac, but, as Streeter pointed out, its dorsal wall will later be that of the gut, whilst its other walls will be those of the yolk sac proper. The alternative to this suggestion of the formation of the yolk sac is that the primary mesoderm does not spread over the ventral surface of the entoderm, and that the cleft is not between mesoderm and entoderm but within the entoderm itself. In opposition to this, however, it must not be overlooked that, at least in the macaque, the cells of the dorsal wall of the cleft are quite different from those of its other walls.

The amnion. As with the yolk sac, the cells of the ventral wall of the amnion are different from those of its other walls (Figs. 8, 10 and 11). Its ventral wall is formed by cells of the embryonic ectoderm; the cells of its other walls are smaller, more widely spaced and more mesodermal in character. Streeter & Heuser (1935) called attention to this sudden transition in the structure of the walls of the amniotic cavity, and suggested that whilst the floor was formed by the ectoderm of the germ-disc proper the remaining walls were trophoblastic in origin.

It has been thought that the human amniotic cavity develops, like that of the bat, by a process of cavity formation in the more dorsal part of the inner cell mass, that the dorsal wall of the cavity is closely adherent to the inner surface of the blastocyst, and that the cells of all its walls are of ectodermal origin. Our ovum, the Hertig ova, and the macaque ovum referred to above, all show the same characters of the amnion (Fig. 11). The cavity is rather

flattened in a ventro-dorsal plane, more so in the other specimens than in ours. The ventral wall is thick and formed by the embryonic ectoderm, the other walls are very much thinner and formed by cells that resemble those of the primary mesoderm. The dorsal wall is not continuous with the inner surface of the trophoblast, but is attached to it only indirectly through the intervention of a few scattered mesoderm-like cells. That the cavity has arisen by cleft formation seems clear, but it is not so certain whether the cleft has been formed within the dorsal part of the inner cell mass or between the inner cell mass and the trophoblast. If the cleft arose within the inner cell mass one would hardly expect to see such a difference between the cells of the ventral wall and the other walls of the cavity, and the amnion would be more closely attached to the trophoblast. If the cleft occurred between the inner cell mass and the trophoblast it would mean the separation of the inner cell mass from the inner surface of the trophoblast and the filling of the gap between the two with primary mesoderm, resulting in the formation of a cavity bounded everywhere, except ventrally, by cells of the primary mesoderm. The latter seems to be the more probable course and would explain the characters of the amnion described above, though it is more accurate to speak of a gradual separation of the dorsal part of the inner cell mass from the inner surface of the trophoblast accompanied by a filling in of the space with mesoderm rather than of any actual cleft formation.

It thus appears that the yolk sac and the amniotic cavity have a rather similar method of development and that, as Heuser (1938) pointed out, the original inner cell mass is responsible for the formation of a small part only of the actual embryo, that is, the germ disc or embryonic ectoderm and a thin film of entoderm on its ventral surface.

SUMMARY

A description is given of a human ovum at the previllous stage of development which was obtained at an autopsy.

The implantation cavity is unusually well defined and free from blood. It lies between and above greatly dilated glands which are almost filled with partly haemolysed blood, and of which one has burst into the uterine cavity. The closing coagulum appears to be derived partly from the contents of this ruptured gland, and partly from the contents of the implantation cavity. The operculum fills the aperture of entry into the uterine mucous membrane; it is an outgrowth from the wall of the blastocyst, and gives the impression of having almost outlived its usefulness.

The blastocyst shows, growing from its outer surface, cytotrophoblast and syncytium; the syncytium is at the stage intermediate between the formation of the 'Implantations-syncytium' and the 'Zotten-syncytium' of Grosser. The blastocyst is a trilaminar vesicle, its outer wall being the trophoblast, its middle the primary mesoderm, and its inner the exocoelomic membrane. The embryo is attached to the basal portion of the blastocyst, and is composed

of an ectodermal embryonic plate, an entodermal plate, and an amniotic cavity. There is no yolk sac.

The origin of the exocoelomic membrane, the yolk sac, and the amnion is discussed. It is suggested that the exocoelomic membrane is derived, like the primary mesoderm, by splitting off from the inner surface of the trophoblast, that, in agreement with Streeter's suggestions, the yolk sac has its dorsal wall derived from embryonic entoderm and its ventral wall from primary mesoderm, that the amnion has its ventral wall derived from embryonic ectoderm and its dorsal wall from primary mesoderm, and that the inner cell mass is responsible for only the embryonic ectoderm, or germ-disc, and a thin layer of entoderm on its ventral surface.

ACKNOWLEDGEMENTS

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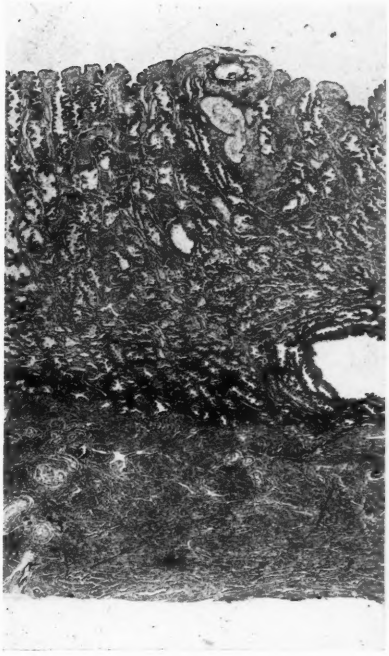
Fig. 1.



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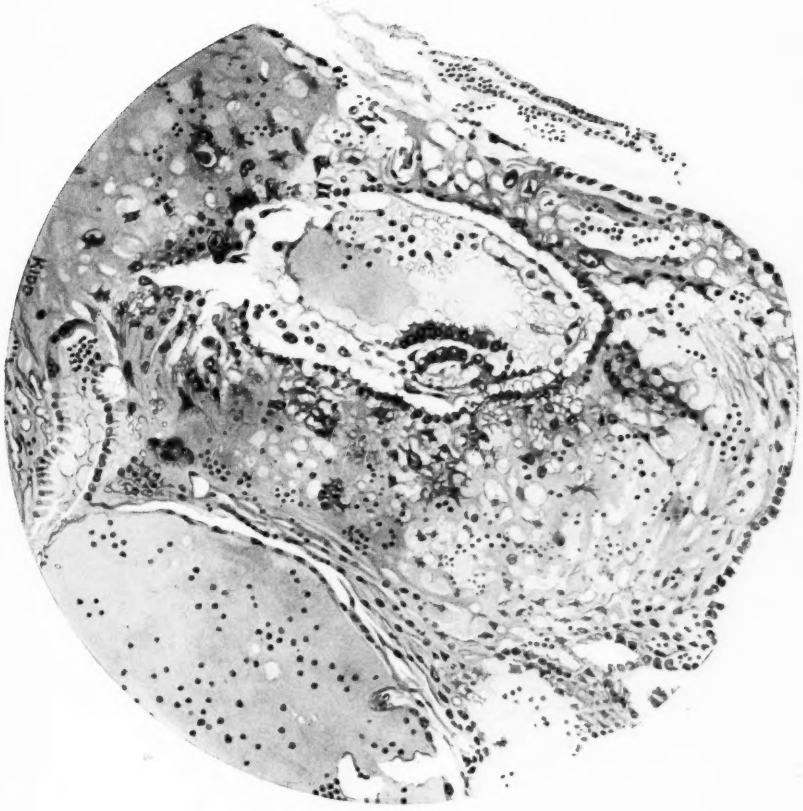

Fig. 3.

Fig. 4.

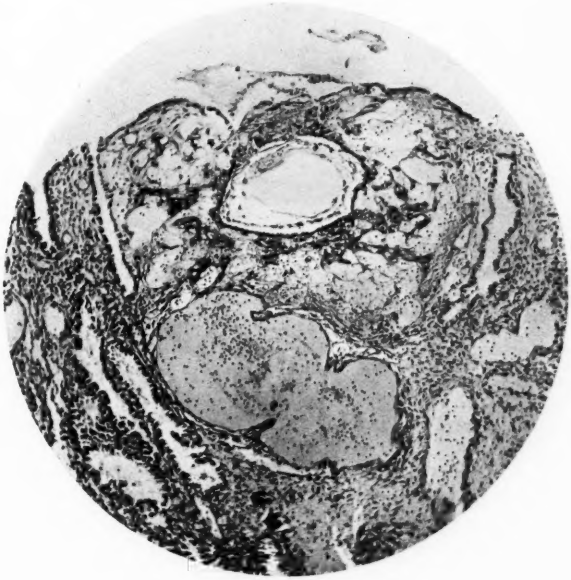

Fig. 5.

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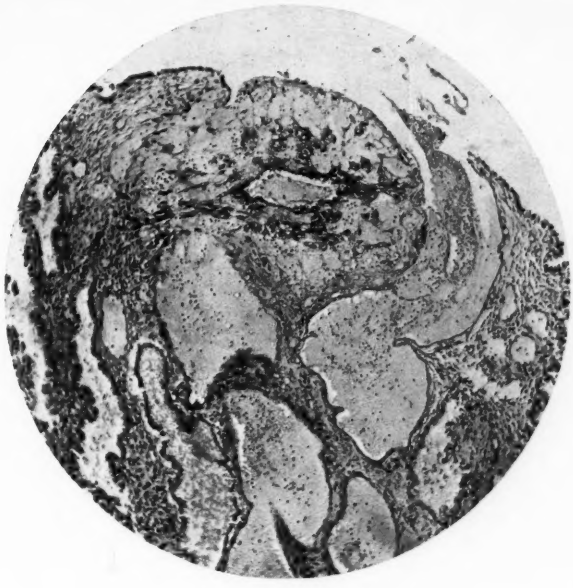

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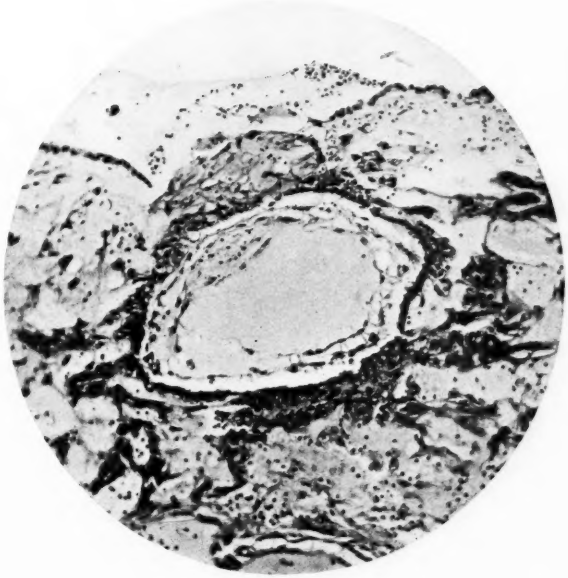

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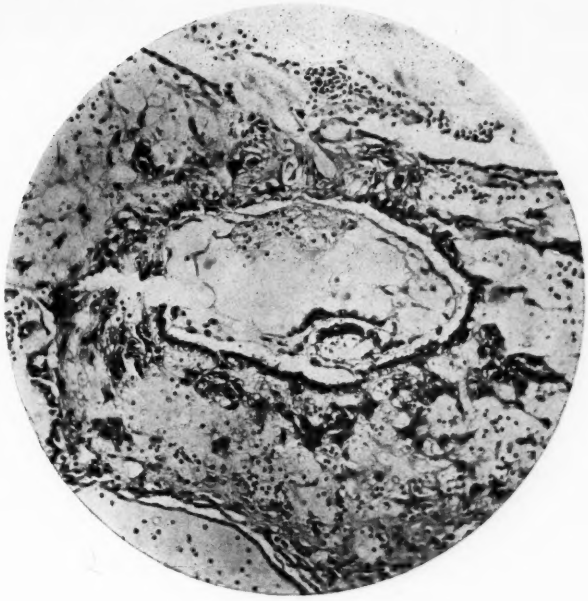

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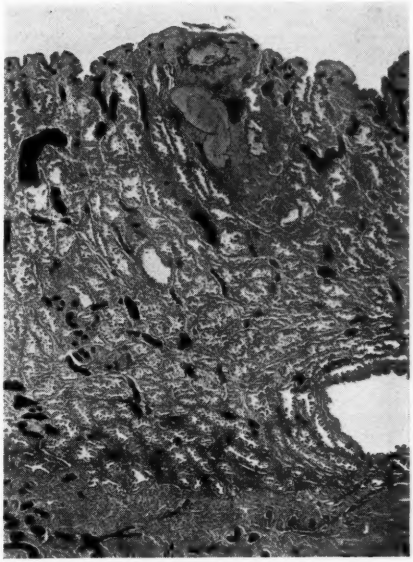

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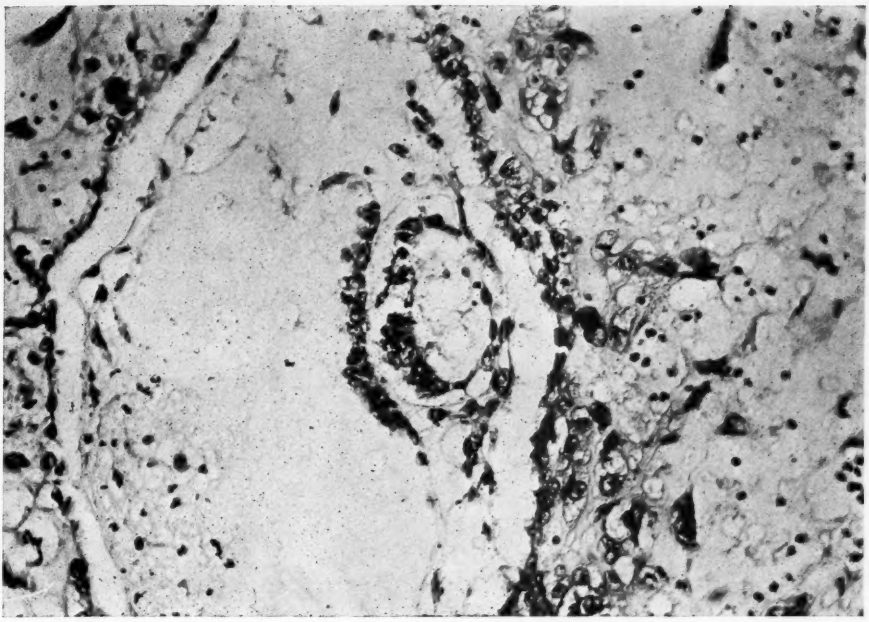

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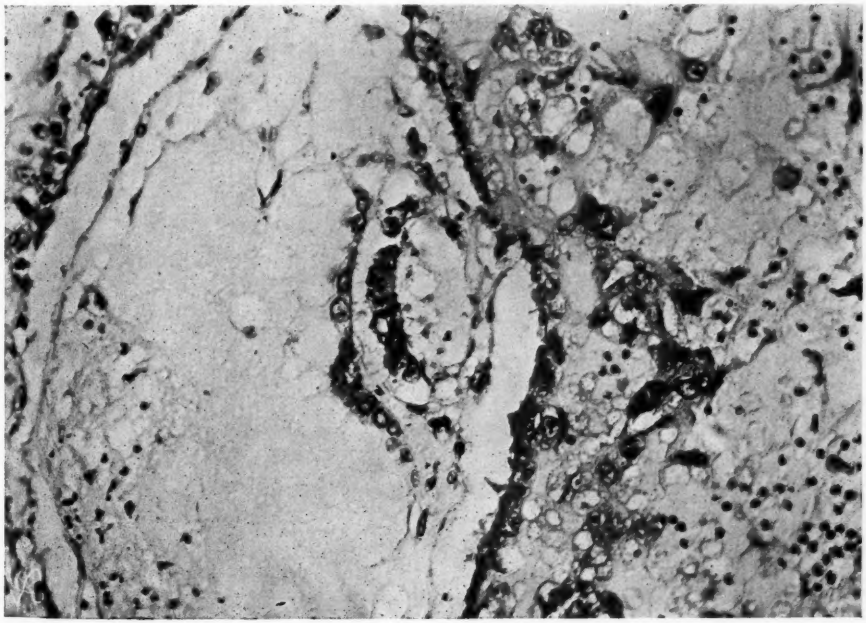

Fig. 10.

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EXPLANATION OF PLATES 1–6

PLATE 1

Fig. 1. The ovum, as seen when the uterus was opened. A wisp of fibrinous material on the summit of a small, dark, circular, congested and elevated area, just to the left of the centre of the photograph, marks the site of implantation. ×1·6 approx.

Fig. 2. A section (slide 25·4) through the uterine wall at the level of the middle of the ovum: this is seen at the top of the section. ×10. (There is an average of 5 sections on each slide.)

PLATE 2

Fig. 3. Coloured drawing of a section (slide 25·2) through the middle of the ovum. ×100.

PLATE 3

Figs. 4, 5. Sections (slides 21·2 and 23·4) through the blastocyst. ×33.

PLATE 4

Fig. 6. Section (slide 27·3) through the blastocyst. ×33.

Fig. 7. Section (slide 24·1) through the blastocyst. ×72.

PLATE 5

Fig. 8. Section (slide 25·2) through the blastocyst. ×90.

Fig. 9. Section through the uterine wall (slide 25·3) on which the blood vessels have been drawn with India ink. ×15·5.

PLATE 6

Figs. 10, 11. Sections (slides 25·2 and 25·4) through the embryo. The amnion is below and the entoderm above. ×200.

EPIPHYSIAL STRUCTURE IN LIZARDS AND MARSUPIALS

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INTRODUCTION

The general arrangement of the secondary centres of lizards, first described by Dollo (1884), has been discussed by Moodie (1908) and Fuchs (1908) who give a good account of the earlier literature. The histological structure has been studied by Heidsieck (1928) in the Gecconidae and Agamidae, and by several authors, especially Wallis (1928) in *Lacerta*. Here a more detailed account of a typical centre will be given and the mechanism of union described, and the description extended to include other groups not previously studied. Some atypical forms will be figured for the first time, particularly that found in *Varanus*, in which cartilage canals have been developed.

In mammals the presumably primitive type of epiphysial centre will be described in marsupials, again for the first time, and the remarkable parallels between mammalian and reptilian evolution in epiphysial structure discussed.

EPIPHYSES OF TYPICAL LIZARDS

In *Agama atra*, which possesses epiphyses of the ordinary type found in lizards, the cartilage at the end of each long bone shows a large secondary centre which divides the articular cartilage (*a.r.*) from the zone of growth (*z.g.*) over the greater part of their extent. In the young bone a zone of undifferentiated cartilage (*z.u.c.*), with the cells collected into small clumps, surrounds the secondary centre. The periphery of the centre itself is composed of calcified cartilage (*c.c.s.c.*), which is seen spreading by narrow processes (*pro.*) among the groups of cells in the undifferentiated zone, so as finally to isolate these groups in the calcified matrix (*i.g.*).

The calcification of the secondary centre always begins in the more central parts of the cartilaginous epiphysis, and is at first widely separated from the articular cartilage, the growth cartilage and the perichondrium (Nauck, 1936, *Lacerta*; Haines, 1940, *Mabouia*). As the calcified area expands at the expense of the undifferentiated cartilage it eventually comes to lie near the perichondrium at one or more points, whereupon the perichondral tissues erode the matrix by wide blunt processes, without at first forming endochondral bone, a stage seen in *Ichnotropis longipes*. In the *Agama* figured the calcification has spread beneath the perichondrium towards both the articular surface and the shaft (*s.p.s.c.*). The perichondral tissues have made their way into the centre by a relatively narrow entrance (*e.t.s.c.*), and have replaced much of the

calcified cartilage by marrow spaces (*m.s.s.c.*), while on the remains of the cartilage endochondral bone (*e.b.*) has been laid down. In the zone of growth the cells lie in well-marked longitudinally arranged clumps, indicating active division, and in the zone of hypertrophy (*z.h.c.*) the cells form cartilage columns (*c.clm.*). The bays of erosion (*b.e.*) and the endochondral bone have the usual reptilian arrangement.

This kind of epiphysial structure is probably found in all groups of lizards in which the secondary centres become ossified, with the sole exception of the Varanidae. This has been confirmed in Gecconidae, Agamidae, Iguanidae,

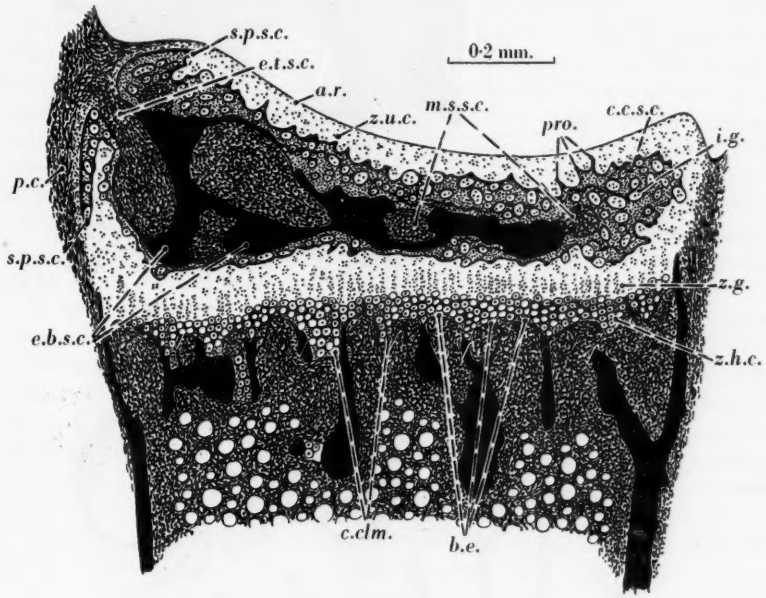

Fig. 1. Head of the radius of *Agama atra*.

Zonuridae, *Heloderma*, Tejidae, Lacertidae, Gerrhosauridae, Scincidae and Chamaeleontidae. The number of points of entry of the perichondral tissues is variable—usually one, but sometimes several. Again, in many epiphysial cartilages there are extra centres starting from intratendinous ossifications, e.g. the epicondyles of the humerus, the surface of the olecranon, the tibial tuberosity, the attachment of the cruciate ligaments to the femur, etc. Finally, as in mammals, some of the cartilages of the metacarpals and phalanges are ossified by extensions from the shaft, instead of by separate secondary centres (Heidsieck, 1928). But the type of ossification in the large limb bones is remarkably constant.

UNION OF SECONDARY CENTRES IN LIZARDS

In later stages the secondary centres grow in volume at the expense of the cartilage, but do not usually cut off the articular cartilage from the growth cartilage completely. Contact of the secondary centre with the perichondrium is always limited and very little perichondral bone is formed, in most cases none at all (Fig. 4). Where independent intratendinous ossification centres

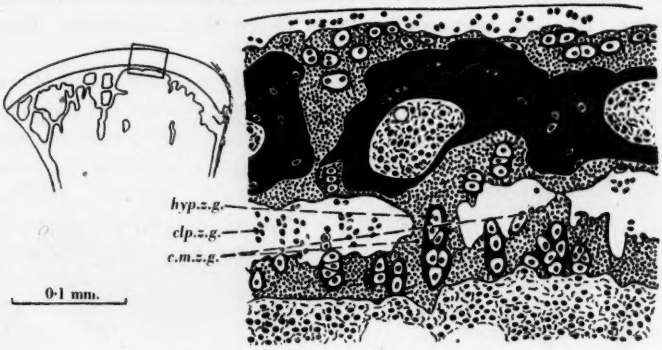

Fig. 2. Upper end of the tibia of *Pseudocordylus microlepidotus*.

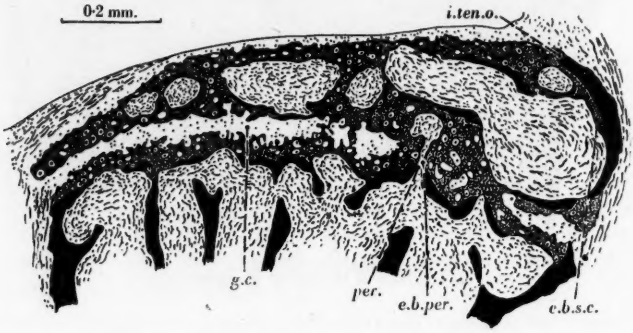

Fig. 3. Upper end of the tibia of *Nucras delalandii*.

have been formed at the insertions of tendons into the epiphysial cartilages, these unite with the main secondary centres before the whole mass unites with the shaft (Fig. 3, *i.ten.o.* and *e.b.s.c.*).

Cessation of growth is first indicated by changes in the growth cartilage (Fig. 2). The cells, while in some regions remaining clumped in a hyaline matrix (*clp.z.g.*), in others all become hypertrophied (*hyp.z.g.*) so that there can be no further formation of cartilage, since the source of young cells is exhausted. The matrix around these hypertrophied cells becomes calcified

(*c.m.z.g.*), so that the calcified cartilage on either side of the growth cartilage is now joined at intervals, and the continuity of the hyaline cartilage broken. Heidsieck (1928) described perforation of the growth cartilage as the earliest stage of union, but it is clear that this co-calcification of the primary and secondary centres precedes the stage of perforation.

In an example illustrating very early perforation of the growth cartilage, of which serial sections were cut (Fig. 3), only one marrow process has actually reached the secondary centre (*per.*). While most of the growth cartilage is still hyaline (*g.c.*) the process has made its way by a narrow canal through the co-calcified region, and has then expanded in the calcified cartilage of the secondary centre, but has as yet made no communication with the marrow spaces of the secondary centre. Along the course of the process some endochondral bone has been laid down (*e.b.per.*), further narrowing the canal as it passes through the growth cartilage. The epiphysial centre itself appears immature, there being a large proportion of uneroded calcified cartilage and little endochondral bone, though the intratendinous bone laid down at the insertion of the patellar tendon (*i.ten.o.*) and the endochondral bone of the secondary centre (*e.b.s.c.*) have already joined. It is clear that the early perforation is carried out entirely by the activity of the marrow of the shaft, that of the secondary centre taking no part in the work.

In mammals there is unfortunately no work published on the histological changes attending the normal union of epiphysial centres. Since the surface of the epiphysial centre towards the shaft usually shows no growth activity, or alternatively active erosion (Payton, 1933; Krompecher, 1937), while the tissues of the shaft are continually eroding the calcified cartilage, it is reasonable to suppose that it is the tissues of the shaft and not those of the secondary centre that are ultimately responsible for union. But the actual occurrence of co-calcification of the shaft and epiphysial centre followed by the growth of marrow processes through the co-calcified areas is, it is believed, described and figured here for the first time in any animal.

As a result of the limited area of erosion of the growth cartilage, isolated fragments of this cartilage may remain as inert masses enmeshed in the trabeculae of endochondral bone for an indefinite period. Heidsieck (1928) considers such a phase normal in lizards, having found it in three species, and it is illustrated here (Fig. 4) in a Cape chameleon. The animal was a fully adult female, with well-developed embryos (the species is viviparous). The remains of the growth cartilage (*is.g.c.*) are completely surrounded by bone (*e.b.*) and therefore incapable of further growth. In other species (Wallis, 1928, *Lacerta*; Heidsieck, 1928, *Ptychozoon*) complete disappearance of the cartilage is described.

In some lizards such as *Phyllodactylus porphyreus*, a small gecko, the secondary centres are never ossified, but remain even in the adult as masses of calcified cartilage (Fig. 5, *c.c.s.c.*). The specimen figured was taken from a fully grown female containing ripe eggs. The growth cartilage (*z.g.*) has

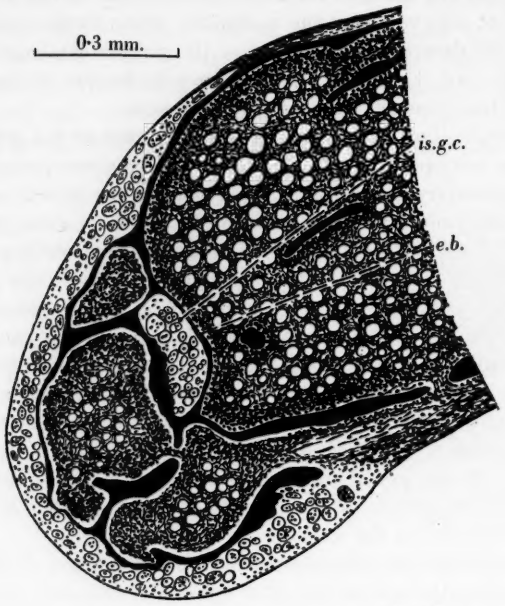

Fig. 4. Lower end of the femur of *Lophosaura pumila*.

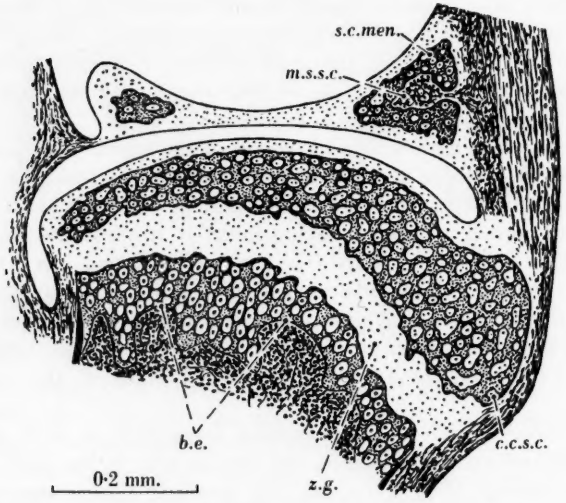

Fig. 5. Upper end of the tibia and semilunar cartilages of *Phyllodactylus porphyreus*.

remained uncalcified, but all trace of special arrangement of the cells has been lost. All the other large long bones show a similar structure of the epiphyses, with massive centres of calcified cartilage, but no marrow or bone. Now at first sight such an epiphysis might appear primitive, for large secondary centres persisting to a late stage of development are found in *Sphenodon* (Haines, 1939), though there they are ossified in the fully adult animal. But it seems more probable that in *Phyllodactylus*, and some other small lizards with a similar structure, the endochondral bone of the epiphyses has been secondarily lost, for in many members of its family the epiphyses are normally developed. The endochondral bone of the shafts has also been reduced, though the bays of erosion (*b.e.*) are found normally on the surface of the cartilage. The semilunar cartilages show a structure similar to that of the epiphyses, but one of the secondary centres (*s.c.men.*) shows a small marrow space (*m.s.s.c.*) eroded by the perichondral tissues. Here again, however, there is no endochondral bone, though a bony structure is usual in this situation in lizards. As regards both the short and the long bones, therefore, *Phyllodactylus* is to be regarded as neotenic in structure rather than as truly primitive. Among mammals a similar neotenic change, the failure in union of the secondary centres at maturity, has been described by Dawson (1929, 1935) in mice and rats.

EPIPHYSIAL STRUCTURE IN *VARANUS*

The Varanidae, now comprising the single genus *Varanus*, contain the largest of living lizards, and are widely separated from all other groups. Parsons (1905), Moodie (1908) and Fuchs (1908) have figured dried and cleared specimens showing secondary centres, but the finer structure has never been described.

In a newly-hatched *Varanus niloticus* the epiphyses contained secondary centres of calcified cartilage (Fig. 6, *s.c.*). The calcification was diffuse, spreading in the matrix between the somewhat hypertrophied cells. This kind of calcification is quite normal in the secondary centres of lizards and is illustrated elsewhere (Haines, 1940), but is unknown in non-reptilian centres.

On the ventral surface the epiphysis overhangs the shaft, as is usual in lizards when the plane of the articular surface is set obliquely to the line of the shaft (Heidsieck, 1928). Between the shaft and this overhanging part of the epiphysis the perichondral tissues give rise to a system of cartilage canals (*can.*). These branch so as to supply the interior of the epiphysis, their terminations (*t.can.*) lying near the articular surface. The canals are somewhat irregular in shape, and occasionally in individual sections islands of cartilage (*is.c.*) are found lying apparently free in the tissue of the canals, but in neighbouring sections they are always found to be joined with the main mass. There are no anastomoses between individual canals, and the numerous blood vessels (*b.v.*) must therefore be 'end artery' systems. Near the canals there is no calcification of the cartilage matrix, and the cells pass by gradual transition from

the loose connective tissue of the canals (*c.t.can.*) through a region of flattened cells (*f.c.*) to the undifferentiated cartilage with small round scattered cells (*r.c.*), and again the transition from these to the hypertrophied cells of the region around the secondary centre is very gradual. In all these characteristics the canals of the young *Varanus* parallel those of mammals (Haines, 1933), and here again the canals appear to be acting as an internal perichondrium forming new cartilage cells at their periphery as well as carrying blood vessels into the interior of the cartilage.

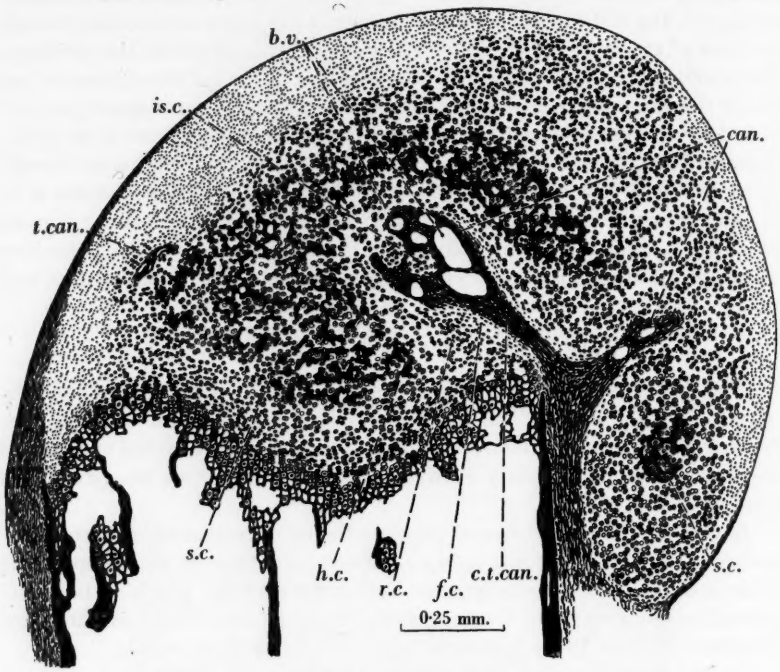

Fig. 6. Lower end of the femur of a newly hatched *Varanus niloticus*.

In an older *Varanus albogularis* the cartilage cells are markedly clumped (*clp.*) and the calcification is now spreading among these clumps so as to surround and isolate them. In many places series of growth lines (*g.l.*) mark the former extent of calcification. The older parts of the calcified matrix are dense (*d.c.m.*) and show splitting, an artifact of decalcification noticed in *Sphenodon* (Haines, 1939). The canals (*can.*) are now sharply bounded on all sides, and there can be no further contribution of cartilage cells to the epiphysial centre, which is heavily calcified and no longer capable of further expansion. The blood vessels (*b.v.*) are active and contain blood corpuscles.

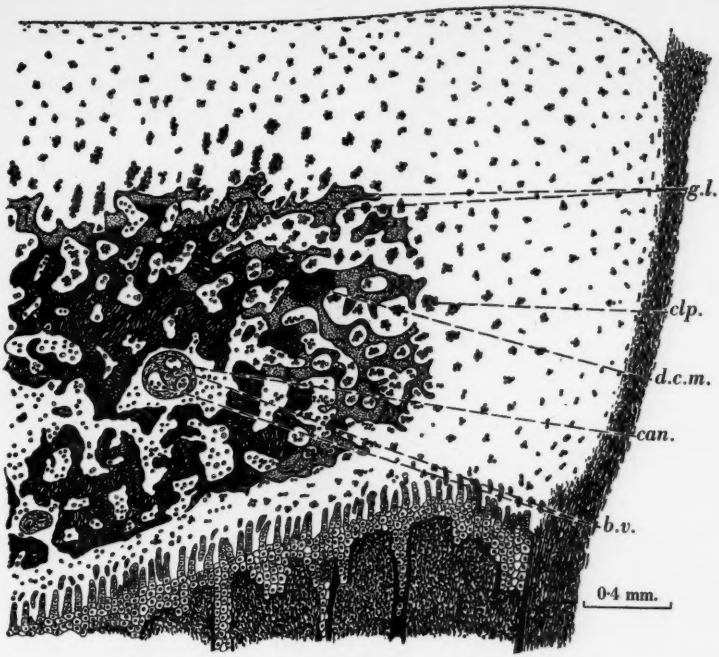

Fig. 7. Upper end of the radius of *Varanus albogularis*.

Fig. 8. Reconstruction in celluloid of the cartilage canals in the upper end of the radius of *Varanus albogularis*.

A reconstruction (Fig. 8) shows the intricacy of the canals at this stage, shortly before ossification begins in the secondary centre. They are more highly developed than any described in mammals, and have fewer points of entry from the perichondrium (only two from the lateral surface in the head of the radius), but otherwise their arrangement is similar to that in mammals.

When ossification of the epiphysial cartilage sets in, it is of course modified by the presence of the cartilage canals. At the lower end of the humerus of a *Varanus exanthematicus* the canals enter from both anterior and posterior surfaces of the bone, those marked *A* (Fig. 9) coming from the one surface and those marked *B* from the other. They ramify through the whole bulk of the cartilage, and there is a specially rich supply to the cartilage overlying the growth zone (*can.z.g.*), but no canal was seen to pierce the growth zone to reach the shaft. The cartilage is calcified as before and shows similar artifacts.

Three secondary centres (*s.c.*) are developed, one for each epicondyle (*ep.med.* and *ep.lat.*) and a central ossification underlying the articular surface, an arrangement paralleled among mammals in the rat (Strong, 1925) and guinea-pig (Harman & Saffry, 1934). The central ossification is nowhere in contact with the perichondrium, being entirely surrounded by cartilage, so that the tissues forming its bone and marrow must have been derived from the tissues of the cartilage canals. Further, since it is supplied by canals from both surfaces, its original position presumably lay between the two sets, which therefore determine the position of the centre. Again, by their spread the centres have cut off the ends of some of the canals that previously traversed the cartilage now replaced by bone, so that these ends now appear to arise from the centres instead of from the perichondrium (Haines, 1933, centrifugal canals; Fig. 9, *cen.can.*). In all these points the Varanidae resemble the mammals.

The epicondylar centres probably began as intratendinous ossifications with secondary spread into the underlying cartilage, similar to those described at the insertion of the quadriceps and triceps, for they have been seen to arise in this way in other lizards. As they spread into the cartilage, however, these centres also gain a blood supply from the cartilage canals.

In later stages the centres join to form a single mass, which has been figured by Fuchs (1908). The canals are now entirely destroyed and the structure is similar to that of epiphyses which have never contained cartilage canals and have been ossified by direct perichondral invasion. Moodie (1908) and Fuchs (1908) are agreed that in *Varanus* the secondary centres do not join the shafts of the long bones, even in old animals.

It has been suggested that *Varanus* is closely related to *Heloderma*, a lizard belonging to another rather isolated group, and Moodie (1908) has claimed a special similarity of arrangement of the secondary centres which would confirm this view. The study here put forward, however, supports the more orthodox view that the Varanidae have no special relationships to other lizards, for *Heloderma* has typical epiphyses without canals.

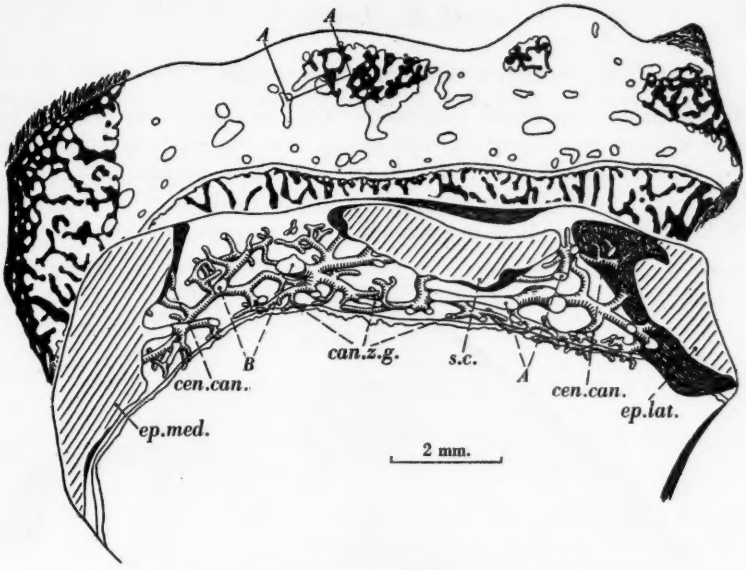

Fig. 9. Section and reconstruction of the lower end of the humerus of *Varanus exanthematicus*.

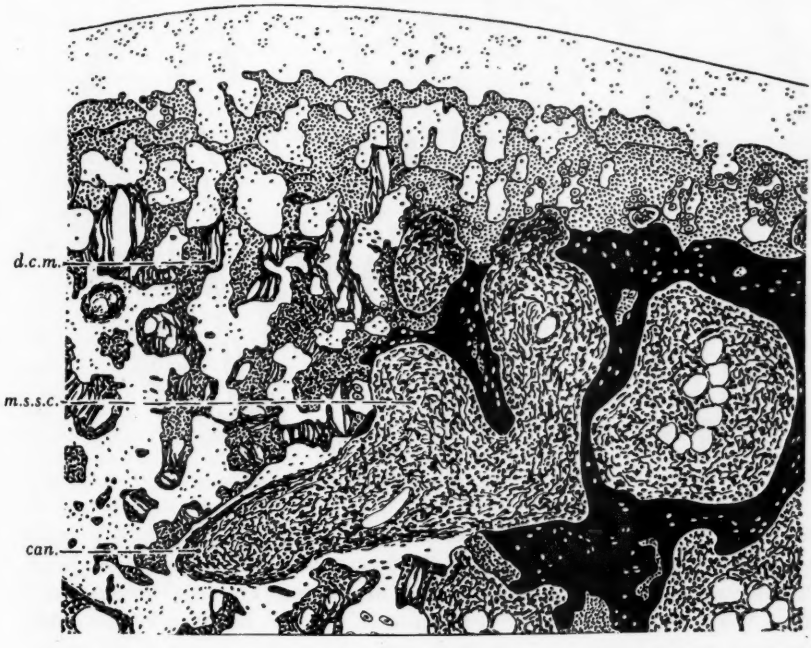

Fig. 10. Enlargement of the portion marked *AA* in Fig. 9.

EPIPHYSES IN MARSUPIALS

In mammals as in lizards ossification of the epiphyses may occur either with or without pre-existent cartilage canals. But the only examples so far known of ossification without canals, the rat and mouse (Haines, 1933), appear to have lost the canals secondarily, owing to the small size of the cartilages, for Bidder (1906) found them in the rabbit. In marsupials, however, the canals

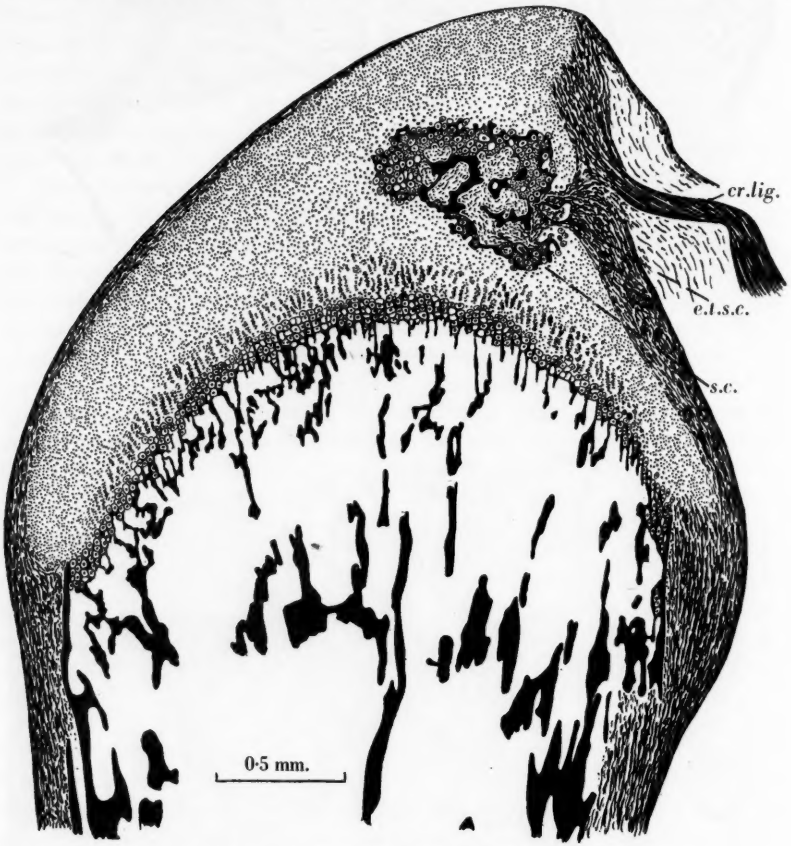

Fig. 11. Lower end of the femur of *Didelphis marsupialis azarae*.

do appear to be primitively absent. In a young *Didelphis* with a small secondary centre in each condyle of the femur, an arrangement found also in the mouse by Johnson (1933), no canals were found (Fig. 11). Indeed, apart from the relatively small size of the area of calcified cartilage and its rapid ossification, the histological picture is very similar to that seen in the typical

lizards. The absence of canals has also been confirmed in *Macropus* and *Dendrolagus*, animals sufficiently large to show canals if these occurred in marsupials.

Thus in the mammals and the lizards a phylogenetic stage of perichondral ossification has passed into a stage of ossification by cartilage canals by one of the most precise parallelisms known in evolution. But whereas only one group of lizards has acquired canals, the vast majority of mammals have them.

If absence of canals is truly primitive in mammals, the monotremes must have acquired them independently. But they are of very peculiar form and their development is unknown.

SUMMARY

1. In typical lizards (all families studied except Varanidae) the secondary centres begin as masses of calcified cartilage which, when they approach the perichondrium, are replaced by marrow and bone.

2. When growth is completed the cells of the growth zone lose their typical arrangement and the secondary centres are joined to the shaft by calcified cartilage. Perforation of the cartilage takes place first through the regions of co-calcification.

3. The failure of ossification or union of secondary centres found in some lizards is a secondarily acquired neotenic feature.

4. The Varanidae show systems of cartilage canals whose structure and fate are very precisely paralleled among the mammals.

5. In marsupials there are no canals and the secondary centres are ossified directly from the perichondrium. This is presumably the primitive arrangement in mammals.

I am indebted to the Thomas Smythe Hughes Fund for a grant towards the purchase of material.

KEY TO LETTERING

a.r.	articular region
b.e.	bay of erosion
b.v.	blood vessel
can.	canal
can.z.g.	canal of growth zone
c.clm.	cartilage column
c.c.s.c.	calcified cartilage of secondary centre
cen.can.	centrifugal canal
clp.	clump of cells
clp.z.g.	clump of cells in growth zone
c.m.z.g.	calcified matrix of growth zone
cr.lig.	cruciate ligament
c.t.can.	connective tissue of canal
d.c.m.	densely calcified matrix
e.b.	endochondral bone
e.b.per.	endochondral bone related to perforation
e.b.s.c.	endochondral bone of secondary centre
ep.lat.	lateral epicondyle
ep.med.	medial epicondyle
e.t.s.c.	entrance of tissues of secondary centre
f.c.	flattened cells
g.c.	growth cartilage
g.l.	growth line
h.c.	hypertrophied cell
hyp.z.g.	hypertrophied cell of growth zone
i.g.	isolated group of cells
is.c.	isolated cartilage
is.g.c.	isolated growth cartilage
i.ten.o.	intratendinous ossification

m.s.s.c.	marrow space of secondary centre	*s.c.men.*	secondary centre of meniscus
p.c.	perichondrium	*s.p.s.c.*	sub-perichondral spread of calcification
per.	perforation	*t.can.*	termination of canal
pro.	process	*z.g.*	zone of growth
r.c.	round cell	*z.h.c.*	zone of hypertrophied cells
s.c.	secondary centre	*z.u.c.*	zone of undifferentiated cells

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THE TERMINATION OF OPTIC FIBRES IN THE LATERAL GENICULATE BODY OF THE MONKEY

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The classical observations made by Henschen (1897, 1898) and Winkler (1912, 1913) on the localization of optic fibres in the lateral geniculate body of the human brain were amplified by Rönne (1913, 1914). This author published a series of clinical cases of alcoholic and diabetic amblyopia which provided definite evidence that the central part of the geniculate body receives fibres of macular origin while the medial and lateral portions are concerned with the reception of impulses from the peripheral zone of the retina. This conclusion was confirmed, on the basis of experimental work on monkeys, by Brouwer (1923) and Brouwer & Zeeman (1925). Brouwer & Zeeman made small circumscribed lesions in the retina and followed the degenerating fibres by the Marchi technique. They found that the macular fibres end in a large central part of the geniculate body, and that fibres from the upper and lower halves of the peripheral retina end respectively in the medial and lateral margins of the nucleus. It is clear, however, that the Marchi method can give only an approximate indication of the site of termination of the optic fibres. In the experiments of Brouwer & Zeeman, for example, it showed nothing of the relation of crossed and uncrossed fibres to alternating cell laminae in the geniculate body—a relationship which had been established in 1920 by Minkowski. Minkowski's conclusions were based on the transneuronal degeneration which follows enucleation of one eye or section of one optic nerve. Le Gros Clark (1932) confirmed these observations on the monkey and first showed that a similar arrangement also obtains in the human brain. The relationship may be expressed simply by the statement that, if the six cell laminae of the lateral geniculate body are numbered from the hilum of the nucleus (Text-fig. 1), crossed retinal fibres terminate in laminae 1, 4 and 6, while uncrossed fibres end in laminae 2, 3 and 5 (Le Gros Clark, 1941). Further proof of this arrangement in the human brain was provided by Hechst (1933).

In 1934 Le Gros Clark & Penman sought for more accurate information on the retinal projection in the lateral geniculate body, using the phenomenon of transneuronal degeneration as an index of the actual site of termination of fibres from different parts of the retina after effecting small circumscribed lesions in the retina. In these experiments it was found that the cell atrophy in the geniculate body is always quite localized and sharply defined, suggesting that the projection of the retina on the lateral geniculate body at least approximates to a point-to-point relationship.

In spite of these studies, nothing is yet known of the actual morphology of the terminals of the optic fibres in the lateral geniculate body of higher

mammals, nor is it known with how many geniculate cells each optic fibre comes into contact. Indeed, the inference that crossed and uncrossed fibres end in different cell laminae has depended hitherto entirely on the indirect evidence of transneuronal degeneration. But this type of degeneration in itself raises new problems regarding the terminal relation of optic fibres and geniculate cells, for it demonstrates that these cells undergo marked and rapid atrophy if the afferent fibres conveying the functional stimulus are sectioned. A slight degree of cell atrophy has been observed in the geniculate body of the cat after section of the optic nerve, but marked and rapid transneuronal degeneration has only been recorded in the monkey and Man. In lower mammals it either does not occur at all, or is so slight as to be not readily detected on direct observation. In the monkey, such transneuronal degeneration does not appear to occur in the ventral nucleus of the thalamus after lesions involving the medial fillet (according to our unpublished observations), and, indeed, it seems doubtful whether it occurs anywhere else in the brain of this animal. Foerster *et al.* (1933) reported a transneuronal degeneration in certain cells of the spinal cord following section of posterior roots, but these observations are perhaps open to a different interpretation, for the cells which were stated to show atrophy were few and scattered, and those who have attempted to plot the position of chromatolytic cells in the spinal cord after section of spinal nerve roots will agree that it is often a matter of great difficulty to distinguish true chromatolysis from the variable appearance of normal cells. Schimert (1938) was unable to confirm the statements of Foerster *et al.* with regard to the alleged transneuronal degeneration in the spinal cord.

The rapid transneuronal degeneration which occurs in the lateral geniculate body of the monkey suggests that the terminal relation between the optic fibres and the geniculate cells is of a particularly intimate nature. The present study was initiated in order to inquire into this question. The only observations hitherto recorded on the mode of termination of optic fibres in the lateral geniculate body are those of Kölliker (1896) on the mouse, and Tello (1904) and Cajal (1911) on the cat. Kölliker merely reports that fibres end in a brush formation. According to Tello the optic fibres enter the hilum of the geniculate body in the cat and ramify in the three cell layers of the nucleus. As soon as a fibre enters its particular layer it divides into a fusiform reticulum which, as he says, resembles a cypress tree. However, the Golgi technique used by Tello is not adequate for demonstrating the precise relation of a single optic fibre to the geniculate cells, nor the actual morphology of the ultimate fibre ending.

MATERIAL AND METHODS

Normal and experimental material was used for the study of the nerve endings in the lateral geniculate body. The experimental material was provided by five monkeys in which one optic nerve had been sectioned, the animals being killed 3, 7, 17, 18 and 46 days after operation.

A variety of silver-impregnation methods were used for the demonstration of nerve fibres and their terminals. Of these the following technique proved very successful in the study of degenerating terminals:

1. Fixation in 10 % formalin—Pyridine 4 drops per 100 c.c. for 6–8 days.
2. Wash in distilled water 12 hr.
3. 0·4 % NaOH 12 hr.
4. Wash in distilled water 12 hr.
5. 10 % $AgNo_3$ 12 hr.
6. 1 min. wash in distilled water.
7. 15 min. in Hortega's silver carbonate.
8. Reduce in 10 % formalin.
9. Clear and mount with or without toning.

Another very useful method which was employed was that of Bielschowsky-Gros, as recommended by Schimert (1938). Frozen sections were also stained with gallocyanin, in order to determine the disposition and pattern of the cell laminae of the geniculate body for comparison with the silver sections.

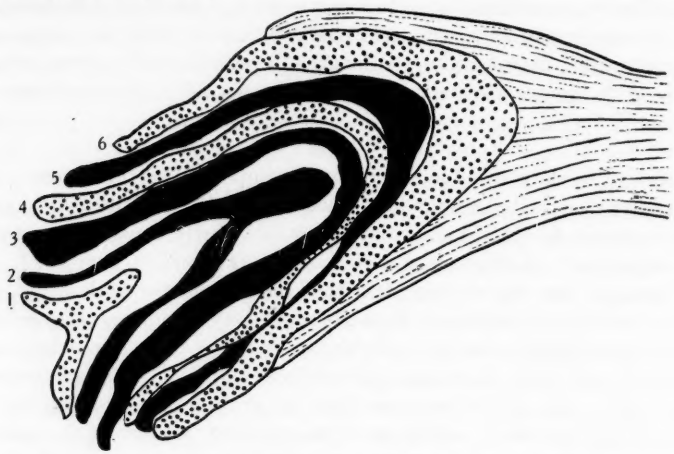
OBSERVATIONS

Although in the normal and experimental material sections were made in a variety of planes, it was found most useful, in order to follow the course of the optic fibres, to cut the geniculate body in a vertical plane along the axis of the optic tract. A diagram of such a section is shown in Text-fig. 1. From the diagram it becomes at once clear that the bulk of the optic fibres do not enter the lateral geniculate body from the hilum; on the contrary, they penetrate the curved cell laminae on their convex aspect, many of them passing through the greater part of the geniculate body in order to reach the cells in the region of the hilum. Silver sections show that, on entering the nucleus, the optic tract splits up into fairly well-defined fasciculi, and from these the individual fibres turn out abruptly to enter the various cell laminae. From their direction it is clear that in order to reach lamina I (bordering the hilum) many of the fibres must pass through all the other cell laminae. It is also clear that the course taken by the entering optic fibres bears no relation to the fibre layers which intervene between the cell laminae of the lateral geniculate body. These fibre layers, indeed, are formed not by the optic fibres but mainly by the geniculo-cortical fibres. This is demonstrated clearly enough in those cases of congenital anophthalmos in which no optic tract fibres are present. In such cases, Weigert sections of the lateral geniculate body show that laminae of medullated fibres are still present in this nucleus (see, for example, Fig. 4 in a recent paper by Whitnall & Norman, 1940).

An examination of the normal geniculate body in silver-impregnated material shows such a density of nerve fibres among the cells of the nucleus that it is quite impossible to identify and follow individual optic fibres to their termination. But this material demonstrated the existence in relation to some of the cells of the geniculate body of what appear to be terminal boutons of

a characteristic type. As shown in Pl. 1, fig. 1, these boutons are in the form of very fine rings. They are by no means easy to find, but where present each ring lies in contact with the cell body and in no instance was it possible to demonstrate a terminal bouton related to a dendritic process. Moreover, a careful scrutiny failed to show any nerve cell in the geniculate body which received more than one terminal bouton.

It seemed probable that these terminal boutons were the end-formations of the optic fibres, but of this no proof could be obtained in normal material. It was therefore decided to see whether after section of the optic nerve the boutons showed any of the characteristic degenerative changes which have been observed to occur in the spinal cord after section of descending tracts (Hoff, 1932; Gibson, 1937).



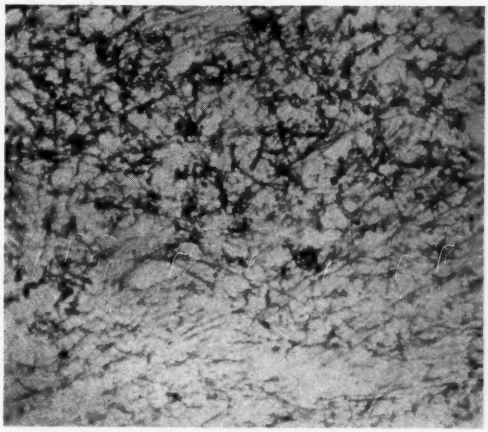
Text-fig. 1. Projection drawing of a sagittal section through the lateral geniculate body of a rhesus monkey, showing the termination of the optic tract and the disposition of the cell laminae. It will be noted that in this plane the hilum of the lateral geniculate body is at its caudal end, while the optic tract fibres penetrate it from its convex aspect.

Experiment O.M. 502. In this experiment the monkey was killed 3 days after section of the right optic nerve. In the right (ipsilateral) geniculate body it is quite evident, even under a comparatively low-power objective, that there are many fibres in laminae 2, 3 and 5 which are swollen and which show a much deeper impregnation when compared with similar fibres in those laminae (1, 4 and 6) which receive crossed optic fibres. With an oil-immersion lens it can be seen that the ring of the terminal boutons is in many instances thickened, somewhat enlarged, and stained more densely than in normal preparations. These changes are also to be observed in the left geniculate body, but they are here found in laminae 1, 4 and 6. The interior of the ring-like boutons in these laminae is often found to be filled with a dense, opaque mass which on close

scrutiny appears to be composed of a very fine neurofibrillar network (Pl. 1, fig. 2). In other instances the boutons have become converted into small solid, black end-bulbs.

Frequently in the affected laminae a fibre is found branching in terminal ramifications, and in such a case the fibre at the site of its division, and also its branches, are conspicuously thickened and very deeply stained (Pl. 1, fig. 3). This appearance, indeed, is a much more obtrusive feature of the affected laminae than the end-bulbs, for although the latter are clearly undergoing degeneration they are at this stage still relatively small.

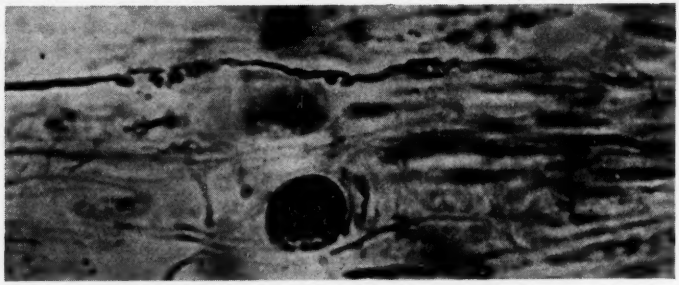
Experiment O.M. 503. In this experiment the animal was killed 7 days after section of the right optic nerve. Lower power examination of silver



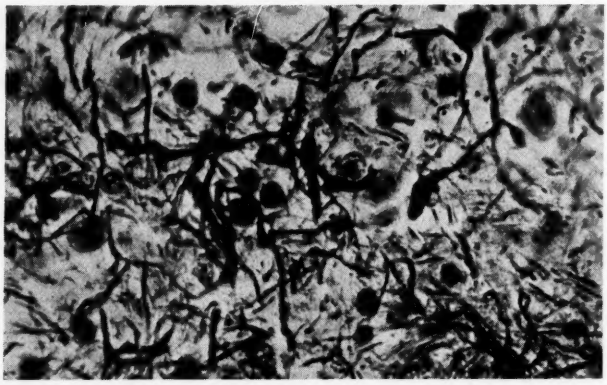
Text-fig. 2. Section through a part of the lateral geniculate body from Experiment O.M. 503, showing above a cell lamina filled with degenerating terminals of optic fibres, while below is a normal lamina. Silver impregnation. ×150.

sections shows that laminae 1, 4 and 6 of the contralateral geniculate body and laminae 2, 3 and 5 of the ipsilateral nucleus stand out in strong contrast with the other laminae by reason of the dense staining of the fibre plexuses which pervade them (Text-fig. 2). With a high-power objective the meaning of this contrast becomes clear. From the optic fasciculi which penetrate the geniculate body, thickened fibres can be seen turning out at right angles to enter the affected cell layers and here undergo a terminal branching. As is seen in Text-fig. 3 the degenerating fibres of the optic fasciculi present an irregular contour and also show an incipient fragmentation. Other fibres are distinguished by a cylindrical swelling in their course (Pl. 1, fig. 4). When an optic fibre approaches the group of geniculate cells in relation to which it is destined to end, it divides into several branches. The mode of branching is shown in the photomicrograph in Pl. 2, fig. 1. In this particular instance, the optic

fibre first gives off two rami and later divides into two branches. Of the latter, one ends in an enlarged end-bulb; the other forms three small branches terminating in smaller bulbs. Each of the end-bulbs is in contact with the body of a nerve cell. In general the boutons of the affected laminae are grossly degenerated, and often appear as large, elongated and densely black bulbs, which form conspicuous objects even under low-power objectives (Text-fig. 4).



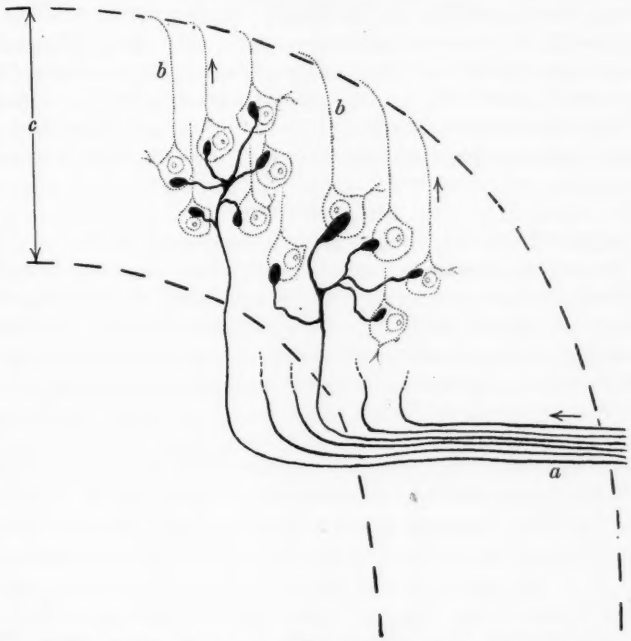
Text-fig. 3. Section of a fasciculus of the optic tract from Experiment O.M. 503, showing a degenerating axone which is thickened and irregularly coiled. ×900.



Text-fig. 4. Section through a cell lamina of the lateral geniculate body from Experiment O.M. 503, showing enlarged degenerating boutons and thickened terminal fibres of the optic tract. ×400.

There is, however, considerable variation in the extent of the degeneration of different end-bulbs, some remaining quite small. Occasionally, also, an apparently normal bouton with a typical ring form can be found in connexion with one of the terminal optic fibres (Pl. 1, fig. 6). Such endings are more frequent in the large-celled laminae (1 and 2). In these laminae the cells are distinguished not only by their size but also by their more numerous dendritic processes and their deeper staining with basic dyes. The functional significance

Text-fig. 5. An enlarged terminal bouton lying in relation to the body of a geniculate cell, from Experiment O.M. 503. ×2000.



Text-fig. 6. Diagram (constructed from a study of the sections of Experiment O.M. 503) showing the termination of optic fibres in relation to the cells of the lateral geniculate body. A fasciculus of optic fibres (*a*) is shown entering the geniculate body from the right. From this fasciculus, individual fibres turn out at right angles to enter their appropriate cell lamina (*c*). Each fibre ends in a spray of 5–6 branches, and each of these terminates in an end-bulb which lies in contact with the body of one geniculate cell. The axones of the geniculate cells (*b*) pass into the fibre laminae of the nucleus and run through these to reach the optic radiations.

of these morphological differences is obscure, for, as far as is known, the connexions of the cells of these laminae are the same as those of the other small-celled laminae.[1] It may be noted, however, that in the large-celled laminae no terminal branching of the optic fibres was observed. The enlarged and solid degenerating end-bulbs provide the only objective evidence here of the section of the optic nerve fibres.

A careful study of the sections from experiments O.M. 502 and 503 leads to the conclusion that (except possibly in the large-celled laminae) each fibre when it has turned out at right angles from the optic fasciculi entering the geniculate body commonly divides into 5 or 6 rami, each of which is related to one geniculate cell. In other words, each of the fibres from the retina is related at its termination to 5 or 6 geniculate cells. It is also to be noted that in no instance was any cell found to be in contact with more than one end-bulb, and that the end-bulb is always in contact with the cell body and not with its dendritic processes (Pl. 1, fig. 6).

In the other experiments (in which the animals were killed 17, 18 and 46 days after section of one optic nerve), the silver preparations provide no further details regarding the mode of termination of the optic fibres. The main optic fasciculi, and also the laminae whose afferent fibres had been interrupted, show an abundance of grossly degenerating axones, and no normal or degenerating boutons are to be found in the affected laminae of the geniculate body. In all these cases, transneuronal degeneration in the geniculate body is conspicuous.

DISCUSSION

The demonstration of typical ring-shaped boutons in the lateral geniculate body of the monkey, similar to those which occur in relation to the motor cells of the spinal cord, at once suggested the possibility of studying the optic terminals by reference to the degenerative processes which they might undergo after section of the optic nerve. The value of degenerating boutons for the determination of the precise site of termination of fibre tracts in higher mammals was indicated by the work of Hoff (1932) on the relation of pyramidal tract fibres to anterior horn cells. In 1937 Gibson reported an experimental study of the 'bouton method' and provided a useful historical survey of the subject. In 1938 Schimert was able by this method to establish the site of termination of the fibres of the vestibulo-spinal tract in the cat. For this purpose he recommended the Bielschowsky-Gros technique of silver impregnation. Schimert considers that the appearance of the end-bulb itself is not always a safe criterion of degeneration, unless it is accompanied by degenerative changes affecting the terminal fibre which carries it. In a recent paper, Phalen & Davenport (1937) put forward some justifiable criticisms regarding the validity of the method, for they point out that normal boutons show considerable

[1] Balado & Franke (1937) have stated their opinion that the cells of the large-celled laminae of the geniculate body project on to the superior colliculi. However, it is a fact, as Poliak (1933) has noted, that all the cells undergo retrograde atrophy following removal of the visual cortex.

variation in their contour, size and appearance. They conclude that it can only be employed successfully for locating synaptic connexions if the degenerating end-bulbs are massive in their extent. Still more recently, Barnard (1940) has strongly criticized the observations of Hoff, Gibson and Schimert. This author was unable to demonstrate any degenerative changes in the end-bulbs related to the cells of the spinal cord. No variation from the normal was observed below the level of the lesion after hemisection of the cord, or after section of the dorsal roots. The present communication deals only with degenerative changes in the lateral geniculate body, and therefore Barnard's paper hardly calls for discussion here. We would only say that we have found some difficulty in appraising this paper (and also some other papers dealing with the same subject) because it lacks the objective evidence of photomicrography. We would, however, suggest that, if Barnard's conclusions should lead anyone to doubt whether end-formations ever undergo the degenerative changes which have been described by previous authors, the simple experiment of sectioning one optic nerve in a monkey should be performed, and the lateral geniculate body examined 7 days later by appropriate and critical silver methods. From the description in the present paper of the boutons in the lateral geniculate body 7 days after section of one optic nerve it will be apparent that the degeneration is massive, for the affected laminae are scattered with a profusion of grossly degenerated end-bulbs. Moreover, their presence is made the more conspicuous by comparison with the alternating laminae in which the boutons remain normal in appearance (see Text-fig. 2). The synaptic connexions of the lateral geniculate body are of course much less complex than those of the cells in the spinal cord, for the geniculate cells probably serve the single function of a relay between the retinal fibres and the visual cortex. Indeed, it is just because of its simple synaptic organization that this nucleus provides much more favourable material than the spinal cord for the general study of the degenerative changes which occur in end-formations after axonal interruption.

The initial degenerative changes in the terminal boutons are a thickening and heavier staining of the ring formation, a slight enlargement, and a filling of the interior of the ring with a silver-reducing substance. As already noted the latter appears to be composed of a close-meshed fibrillar network. It is our impression, in agreement with the opinion previously expressed by Bielschowsky (1935), that in normal boutons the interior of the ring is filled with an extremely fine membrane of neurofibrillar structure—so fine that it is usually difficult to detect. If this is so the 'solidification' which occurs in degenerating boutons is simply due to a thickening of this membrane. As degeneration proceeds the boutons become markedly enlarged to form round, oval and elongated fusiform bodies staining a dense black.

It is of some importance to note that the initial process of degeneration which follows interruption of the optic fibres is not confined to the boutons; it also affects the fibre at the site of its terminal division as well as its telodendritic processes. These become irregularly swollen and stain deeply with

silver impregnation. Indeed, in the earlier stages this degenerative change is much more conspicuous than that which affects the boutons themselves. Moreover, it serves to bring into prominence the form and disposition of the terminal branching of the optic fibres and allows this branching to be seen in the midst of the profuse plexus of fibres which pervades the whole of the geniculate body. In a normal silver preparation it is quite impossible to distinguish this end-formation.

It should be emphasized that the boutons in the normal geniculate body of the monkey are difficult to find in comparison with those of the spinal cord, and they appear to be much fewer than the enlarged bulbs in the degeneration experiments. It seems that this is probably due to the fact that (1) only a single bouton is related to each geniculate cell, and (2) their minute size and light staining, as well as the fact that they can only be defined in certain planes of section, make them difficult to detect. On the other hand, while we have the impression that the enlarged bulbs of the experimental material represent a degenerative process occurring in normal ring-shaped boutons, this does not exclude the possibility that they may also be produced by a local thickening of free terminals. We can only say, however, that such free terminals were not visible in the silver preparations. In any case, it may be observed that the precise mode of formation of the enlarged end-bulbs does not affect the main thesis of this paper, which is concerned with the terminal relation of optic fibres to geniculate cells.

A study of the experimental material makes it evident that the optic fibres penetrate the lateral geniculate body in an antero-posterior direction in discrete fasciculi. From the latter individual fibres turn out at right angles to enter their appropriate cell laminae. With the possible exception of the large-celled laminae, each fibre ends by breaking up into a spray of five or six fine branches each of which ends in a terminal bouton in contact with the body of a geniculate cell (Text-fig. 6).[1] Moreover, it seems probable that no cell in any of the laminae of the geniculate body receives more than one bouton. Thus the cells of the lateral geniculate body stand in strong contrast to the motor cells of the spinal cord which have multiple contacts with terminal boutons.[2] Indeed, Barr (1940) has estimated that in the spinal cord of the cat each single anterior horn cell may have in contact with it as many as a thousand terminal boutons. This is to be explained partly by the fact that the motor cells of the spinal cord are served by many different fibres conveying many different types of impulse. On the other hand, as already noted, it seems that the cells of the lateral geniculate body have one function only to perform—to serve as simple relays for retinal impulses on their way to the visual cortex. It may be noted

[1] We cannot affirm from our preparations that the relation of one optic fibre to 5–6 cells in the lateral geniculate body is invariable; but this seems at least to be the common arrangement.

[2] For purposes of comparison, we have prepared normal control material of the spinal cord, medulla and cerebellum, using the same silver techniques as those employed for the lateral geniculate body. In all these sections we have been able to demonstrate terminal boutons and end-formations corresponding precisely with the classical descriptions given by previous authors.

that another example of a single relay station is provided by the trapezoid nucleus in the hind-brain, which appears also to serve only one afferent system.

The only demonstrable boutons in the lateral geniculate body are those of the optic fibres. This raises the interesting question whether the geniculate cells do in fact receive any other afferent fibre systems. The speed with which transneuronal degeneration occurs in the lateral geniculate body (it is already evident in experiment O.M. 503, 7 days after section of the optic nerve) suggests that this is probably not the case, for it is difficult to explain such a rapid effect except on the basis of the removal of all the afferent stimuli on which the functional activity of the cells depends. The only other afferent fibres to the lateral geniculate body which have hitherto been described are descending connexions from the occipital cortex. The existence of such cortico-geniculate fibres seems to have been accepted by several authors as a part of a general cortico-thalamic system whereby the various thalamic nuclei are brought under the functional control of those areas of the cortex to which they project sensory impulses. In regard to the lateral geniculate body, at least, the evidence for such a descending connexion is meagre.

Biemond (quoted by Walker, 1938) described corticogeniculate fibres in the rabbit on the basis of Marchi preparations, and Mettler (1935), with the same technique, affirms their presence in the monkey. On the other hand, Poliak (1932) doubts their existence in the monkey, and Barris (1935) was quite unable to detect them in the cat. Clearly this is a matter which demands further investigation with a technique which is more critical than the Marchi method. It may be observed that, if the optic fibres are the only afferent fibres of the lateral geniculate body, and no other synaptic connexions exist, it is difficult to understand the purpose of the dendritic processes of the geniculate cells for, as we have seen, the optic terminals end in contact with the cell body, and the existence of intercalated neurones in the nucleus seems to be excluded by the total degeneration of the geniculate body following removal of the visual cortex. It is possible, however, that the dendritic processes of the geniculate cells may receive collaterals from the geniculo-striate fibres, such as have been described by Tello (1904) in the cat. As already mentioned, in O.M. 503 we were able to find occasional end-bulbs of apparently normal appearance, and it might seem possible that these are the terminations of a fibre system other than the optic tract. We think that this is almost certainly not the case, however, for it was generally possible to trace such end-bulbs in connexion with a main optic fibre. Moreover, further evidence that they belong to fibres of retinal origin is provided by the fact that all the end-bulbs related to the bodies of the appropriate geniculate cells have disappeared in the experiment in which the optic nerve was cut 17 days before death.

The inference that (with the possible exception of the large-celled laminae) the receptive unit of the lateral geniculate body commonly consists of a small

and discrete group of 5–6 cells is reflected in the cytoarchitecture of the nucleus as it appears in methylene blue preparations, for it is frequently to be observed that the cells tend to occur in clumps of about the same number. It also is in approximate harmony with the earlier observations of Cajal (1911) and Tello (1904) on the lateral geniculate body of the mouse and cat; in these animals each optic fibre was found to form a plexus of terminals in the meshes of which 6–8 cells are embedded. Neither of these authors described terminal boutons; indeed, Cajal (1935) specifically denies their existence in the lateral geniculate body. But they both employed the Golgi method of impregnation, a method which is usually not adequate for the demonstration of terminal boutons. In addition, Cajal confined his studies to young animals and he points out that in the spinal cord the small discrete end-bulbs are late formations, being preceded in development by a diffuse reticular termination. Further, it is possible that the relation of optic fibres to the geniculate cells may be different in the mouse and cat, related to the fact that transneuronal degeneration in the lateral geniculate body of lower mammals either does not occur or is relatively slight. It may be noted that Cattaneo (1923) described reactive phenomena in the lateral geniculate body of the rabbit 7 days after section of an optic nerve, in the form of end-plates, annular end-formations and boutons. However, he apparently did not study the normal geniculate body of this animal, nor was he concerned with the nature of the normal terminations of the optic fibres in the nucleus.

In a comprehensive monograph on the lateral geniculate body, Balado & Franke (1937) state that in Man the number of cells in the lateral geniculate body is about the same as the number of optic fibres. If this is so, the numerical relation of fibres to cells is very different to that which has been observed in other mammals. However, it is not possible to assess the accuracy of their statement, for Balado & Franke give no details of the methods they used to enumerate the cells in the lateral geniculate body. Also, for a fibre estimate they depended on a count of fibres in the optic nerve although, of course, many of these fibres are destined for the mid-brain and do not end in the lateral geniculate body.

One of the results of this study of the lateral geniculate body has been to provide the first *direct* evidence that crossed and uncrossed retinal fibres end in different cell laminae.[1] That is to say, crossed fibres end in laminae 1, 4 and 6, while uncrossed fibres end in laminae 2, 3 and 5.

SUMMARY

1. Seven days after section of one optic nerve in the monkey, the corresponding laminae in the lateral geniculate body are filled with thickened terminal fibres and enlarged and deeply staining boutons. These laminae therefore stand out in strong contrast with the normal laminae which receive the terminal fibres of the intact optic nerve.

[1] This observation has already been recorded by one of us (P. G. 1940).

2. It is established that each main optic fibre commonly terminates in a spray of 5–6 branches, and therefore in relation to 5–6 geniculate cells. The boutons of the degenerated optic fibres lie in contact with the cell body, and no geniculate cell is related to more than one bouton.

3. By the study of the degenerating terminal fibres and boutons, direct evidence has been provided in confirmation of the conclusion, previously based on transneuronal atrophy, that crossed optic fibres end in laminae 1, 4 and 6 of the lateral geniculate body, while uncrossed fibres end in laminae 2, 3 and 5.

Grateful acknowledgement is made by one of us (P. G.) for the assistance of a grant from the Nuffield Committee for the Advancement of Medicine.

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EXPLANATION OF PLATES 1 AND 2

Plate 1

Fig. 1. A terminal bouton of normal appearance in the lateral geniculate body. ×1500.

Fig. 2. A degenerating bouton from the lateral geniculate body of Experiment O.M. 502. The bouton is slightly enlarged, and the interior of the ring is filled with a fibrillar substance. ×1850.

Fig. 3. The axone terminal of an optic fibre from Experiment O.M. 502. Note the thickening of the fibre at the site of its division. From here, fine terminal branches can be seen proceeding. ×630.

Fig. 4. A degenerating axone in a fasciculus of the optic tract in Experiment O.M. 503. ×900.

Fig. 5. Degenerating terminal boutons in the geniculate body of Experiment O.M. 503. On the left, a bouton is seen lying in contact with the cell body of a geniculate cell. On the right two terminal branches of an optic fibre are seen ending in degenerating boutons. ×400.

Fig. 6. Two cells in the geniculate body of Experiment O.M. 503. In relation to the cell below is an apparently normal bouton, while a solid and irregularly shaped degenerating bouton lies in contact with the cell above. ×1350.

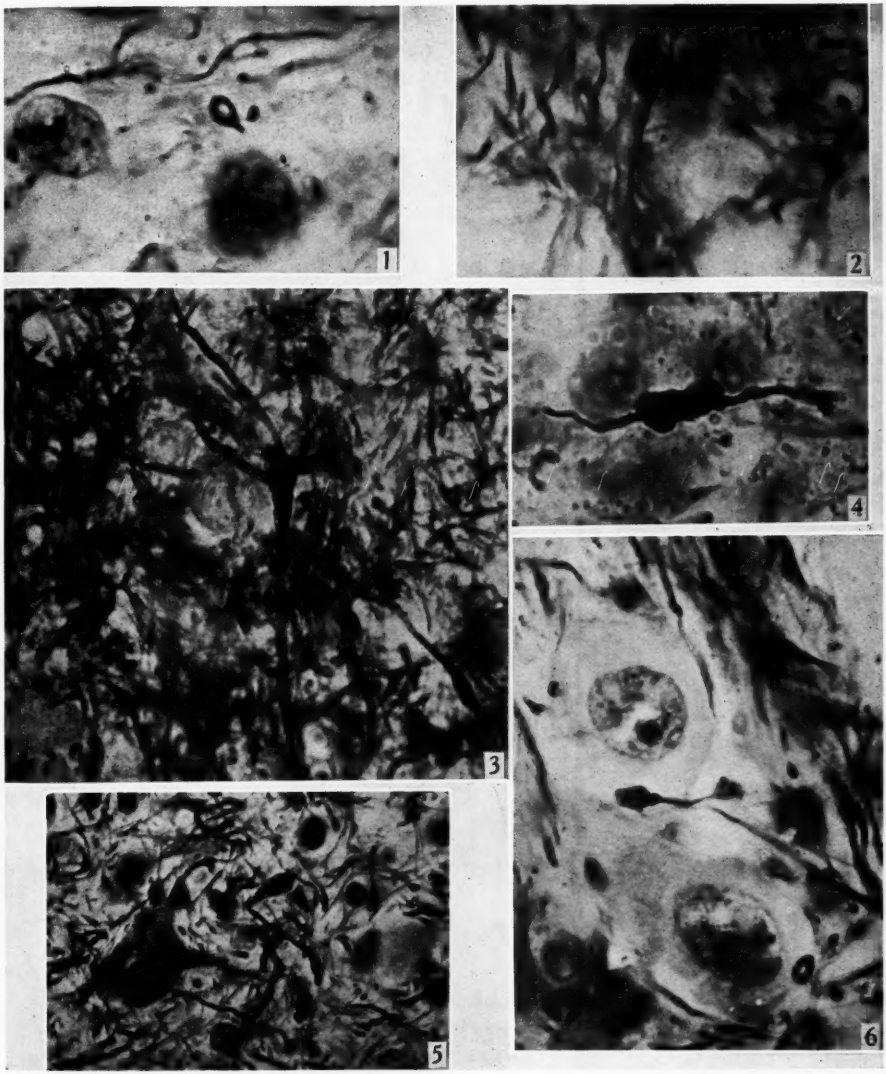
Plate 2

Fig. 1. The degenerating terminal of an optic fibre from Experiment O.M. 503. Note the thickening of the fibre at the point of its division. The fibre divides into six terminal branches, and enlarged terminal boutons are seen on three of these. ×1800.

Fig. 2. Degenerating terminal optic fibres in the lateral geniculate body of Experiment O.M. 503. Note the irregular swelling of the fibres. ×1400.

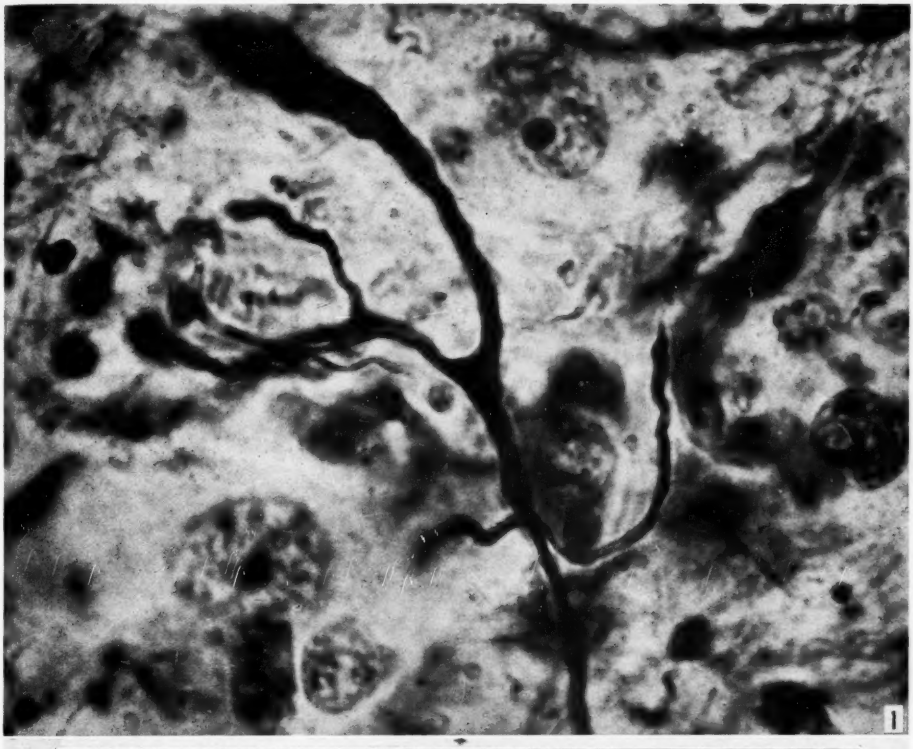
ADDENDUM

Since this communication was sent to the press we have seen a recent paper by J. L. O'Leary on the lateral geniculate nucleus of the cat (*J. comp. Neurol.* **73**, 1940, 405). In this animal it was found that 'within a cellular lamina the terminal arborizations overlap each other ("partially shifted overlapping") so that each principal cell makes synaptic contacts with the terminals of several different optic tract fibres'. If this is the case, it appears to constitute a significant difference from the condition in the geniculate body of the monkey. It is to be noted, however, that O'Leary's observations (like those of Cajal and Tello) were made on Golgi preparations of normal material, so that they are not directly comparable with our own observations which are based on technical methods designed to show directly the relation of optic terminals to geniculate cells. The application of the Golgi technique to the lateral geniculate body of the monkey is limited by the lack of adequate and appropriate material in our laboratory, and such preparations as we have hitherto made fail to show a satisfactory impregnation.



GLEES AND CLARK—TERMINATION OF OPTIC FIBRES IN THE MONKEY

UNIV. OF MICH.



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AN EXPERIMENTAL INVESTIGATION OF THE VISUAL SYSTEM IN THE PHALANGER, *TRICHOSURUS VULPECULA*

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Many experimental or clinical studies have been made on the mammalian visual system but they have been confined principally to four Orders—the marsupial Polyprotodontia (Virginian opossum), the Rodentia (rabbit and rat), the Carnivora (cat and dog) and the Primates (monkey, chimpanzee and man).

The Virginian opossum (*Didelphys virginiana*), a very primitive mammal, crepuscular and arboreal in habit, is the only marsupial in which an experimental study of the visual system has hitherto been made (Bodian, 1935, 1937). In the main the results obtained in this animal are similar to those for other mammals. The present paper deals with the phalanger (*Trichosurus vulpecula*), an arboreal and nocturnal diprotodont marsupial whose nervous system is in general more highly developed than in the polyprotodont marsupials (Goldby, 1939, 1940). It seems of some importance to be able to compare the optic system of such a mammal, not only with that of the more primitive Polyprotodontia, but also with that of the higher placental mammals. For this purpose a preliminary study of the normal anatomy of the optic centres (including the visual cortex) has been made, followed by a more detailed investigation of these centres and their connexions, using the different degeneration techniques.

MATERIAL AND METHODS

The animals used were all healthy adult specimens. In the investigation of normal material several brain dissections were made, followed by a histological study based principally on two complete series of sections. Both series were cut in celloidin, one sagittally at 30μ and the other transversely at 40μ; in each, alternate sections were stained by Weil's modification of the Weigert method and by toluidin blue. A number of incomplete series were also studied; these included several transverse series cut in celloidin at 30–40μ and stained with toluidin blue, and a practically complete series cut in paraffin at 15μ and having alternate slides stained by the Davenport silver method and by cresyl violet.

In the experimental study twelve animals were used. Operations were performed with the usual aseptic precautions under ether anaesthesia. In four

[1] This work has been carried out during the tenure of a David Murray Scholarship from the University of Adelaide.

animals the right eye was enucleated; in each of the remaining eight a lesion was made affecting the visual cortex of the right hemisphere. Cortical lesions were made with either a diathermy apparatus or a small sharp-edged scoop; they varied in size and position in each specimen. Using a camera lucida apparatus, all lesions were drawn as accurately as possible from the complete brain and checked by microscopical examination. It was found that most lesions involved a varying amount of subcortical white matter (particularly those made with the diathermy), and in such cases an estimate was made of the total area affected by the lesion. A dissection showing the direction of the fibres of the corona radiata (cf. Loo, 1930, Fig. 11) facilitated the mapping of this area.

After their allotted survival period all animals were killed with coal gas, injected with 10% formalin, and the brain removed and placed in 10% formalin for at least 24 hr. After cutting into blocks of suitable size all brains (except the one destined for the Davenport silver method) were embedded in celloidin. Transverse serial sections (30–40 μ) were cut through the required regions, and as a routine procedure every fourth section was stained and mounted; where necessary, additional sections were prepared for examination.

Drawings of gross material were made either with the aid of photographs or with a camera lucida apparatus. An Edinger projection apparatus was used for drawing the sections. All measurements reported are the average of a number of readings made on formalin-fixed and celloidin-embedded material. It should be mentioned that in paraffin-embedded brains much greater shrinkage occurs, and measurements made on paraffin material cannot be compared with those from celloidin sections.

For the Marchi technique the animals were allowed to survive their operation by 3 weeks. In two of these specimens the right eye had been enucleated, in the other two a lesion had been made in the visual cortex.

For the transneuronal technique two phalangers were allowed to survive for 100 days after the right eye had been removed. Sections from one of these were stained with toluidin blue; those from the other (which was cut in paraffin) were stained by the Davenport silver method on the slide. Thin longitudinal and transverse sections of the optic nerves stained by the Davenport method were prepared from both these specimens.

For retrograde degeneration from the cortex six animals were allowed to survive for periods varying from 40 to 100 days after a lesion in the visual cortex. Toluidine blue was used as a routine stain for these specimens, but a few sections were stained by Weigert's method. There were also available for study other toluidin blue and Marchi series prepared from brains in which extensive cortical lesions (not including the visual cortex) had been made.

NORMAL ANATOMY

The chiasma

The optic nerves and chiasma present no unusual features, and all the fibres present appear to be myelinated. The chiasma is situated ventral to a well-marked recess of the third ventricle; related to its dorsal surface is the supra-chiasmatic nucleus (nucleus ovoideus of Bodian, 1940), and more laterally the supra-optic nucleus (nucleus tangentialis pars supra-optica of Bodian).

The supra-optic commissures are very similar to those described in the opossum by Tsai (1925) and Bodian (1940). Ganser's commissure, which is situated a little posterior and dorsal to the chiasma, consists of fine myelinated fibres which cross immediately beneath the ventricle and then ascend steeply to be lost in the subthalamus. The larger Meynert's commissure lies in contact with the posterior surface of the chiasma, and some of its fibres run along the medial border of the optic tract where they can be distinguished from the optic fibres by their finer myelination. They appear to enter the zona incerta, and it is probable that many of them reach the subthalamic nucleus. The fibres of Gudden's commissure cannot be distinguished with certainty either in the optic chiasma or tracts. A few fibres leave the optic tract at the dorsolateral border of the cerebral peduncle and enter the medial geniculate body; these are probably fibres from Gudden's commissure. The fasciculus supra-opticus (tractus olfactotuberalis of Tsai) is a small finely myelinated fibre bundle closely related to the dorsal surface of the chiasma. Its extent and connexions seem similar to those described in the opossum (Bodian, 1940).

The optic tract

The optic tract has its usual course round the cerebral peduncle, at the dorsal border of which it is joined, on its medial side, by fibres coming from the peduncle. It then divides (Fig. 1), just ventral to the lateral geniculate body, into a lateral and a medial division, corresponding to the mesencephalic and diencephalic divisions of Tsai (1925). The larger lateral division forms a well-marked layer lying for the most part over the outer surface of the lateral geniculate body and the nucleus lateralis posterior of the thalamus; the most posterior fibres, however, run dorsally over the outer surface of the medial geniculate body. On approaching the pretectal region the fibres of the lateral division dip just beneath the surface to run through the large-celled nucleus of the optic tract and the superficial part of the pretectal nucleus, after which they enter the superior colliculus. The fibres of the medial division run through the lateral geniculate body (ventral and dorsal nuclei) and the nucleus lateralis posterior of the thalamus; some of them join the lateral division in the pretectal region, but most of them are more deeply situated. Fibres from both divisions of the optic tract appear to end throughout the dorsal nucleus of the lateral geniculate body and also in the stratum opticum and (to a smaller

extent) in the stratum griseum superficiale of the superior colliculus. No definite evidence for the termination of optic tract fibres in the ventral nucleus of the lateral geniculate body, the pretectal nucleus, or the large-celled nucleus of the optic tract was found in normal material.

Two small accessory optic tracts are present. The anterior accessory optic tract leaves the posterior border of the main tract as it reaches the cerebral

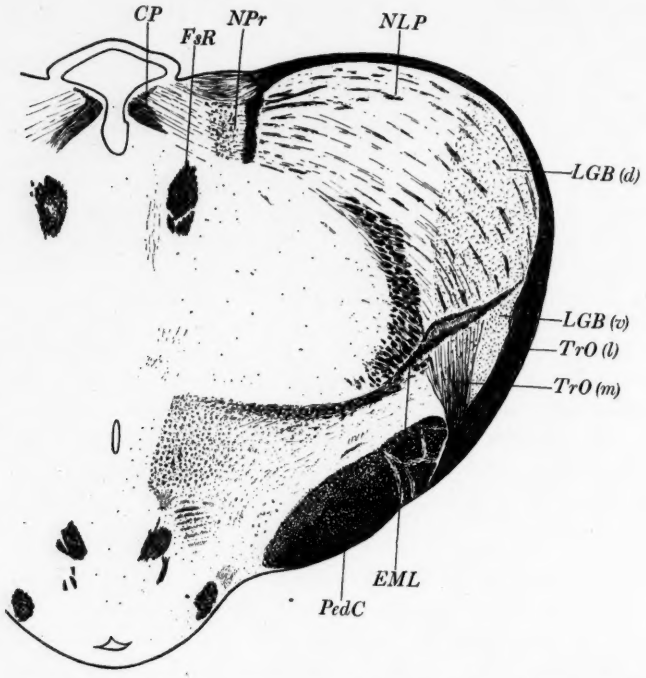

Fig. 1. A drawing of a transverse section through about the middle of the lateral geniculate body; myelinated fibres stained. Magnification ×10. *CP*, posterior commissure; *EML*, external medullary lamina; *FsR*, fasciculus retroflexus; *LGB* (*d*), lateral geniculate body, dorsal nucleus; *LGB* (*v*), lateral geniculate body, ventral nucleus; *NLP*, nucleus lateralis posterior of the thalamus; *NPr*, pretectal nucleus; *PedC*, cerebral peduncle; *TrO* (*l*), optic tract, lateral division; *TrO* (*m*), optic tract, medial division.

peduncle and passes caudally along the medial border of the peduncle to the region of the nucleus opticus tegmenti. The transverse peduncular tract leaves the posterior border of the main tract just dorsal to the lateral border of the peduncle and curves ventrally over the peduncle to join the anterior accessory optic tract a little anterior to the nucleus opticus tegmenti. Bodian (1940) has reported that in the opossum this tract is made up chiefly of the superficial fibres of the tractus geniculatus descendens. In the phalanger, however, the

optic tract appears to contribute most of the fibres found in the transverse peduncular tract.

Optic centres

The normal anatomy of the pretectal nucleus, the large-celled nucleus of the optic tract and the lateral geniculate body, has been described shortly by Goldby (1940). He has illustrated the marked lamination pattern which is present throughout the dorsal nucleus of the lateral geniculate body except for its ventral quarter where laminae 1, 2 and 3 become intermingled.

The superior colliculus is a well-developed rounded body lying between the smaller inferior colliculus posteriorly and the thalamus anteriorly; it is hidden by the overlying occipital lobe of the hemisphere. The internal structure of this body shows no significant differences either from that described in *Didelphys virginiana* by Tsai (1925) or from the general description given by Kappers *et al.* (1936).

The nucleus opticus tegmenti is a small nucleus situated ventromedial to the cerebral peduncle at the medial end of the substantia nigra and a little anterior to the point of exit of the oculomotor nerve; it extends over some twenty transverse sections (30 μ). Its cells are compactly arranged; some are polygonal, others fusiform in shape. In normal material the connexions of this nucleus cannot be definitely established. The anterior accessory optic tract and the transverse peduncular tract certainly become lost near it.

In accordance with Brodmann's (1909) observations in other mammals, a six-layered pattern is present in most parts of the cerebral cortex of the phalanger. As usual, the area striata is characterized by a well-developed fourth layer. In determining the extent of this area (Figs. 2, 3) it was found that a limiting sulcus (intercalary and calcarine) is present marking the medial boundary; the lateral boundary can only be determined microscopically and is marked by a fairly abrupt narrowing of layer IV, with a corresponding broadening of layer V. The laminae of the visual cortex and their constituent cells are as follows (Fig. 4):

(i) The molecular layer (0·20 mm.), in which a few small neurones are present.

(ii) The outer granular layer (0·06 mm.) is narrow and ill-defined; its cells are of medium or small size and polygonal in shape. They stain deeply.

(iii) The outer pyramidal layer (0·60 mm.) is a broad but not compact layer with its cells less numerous towards the inner surface. Medium-sized pyramidal cells are characteristic of this layer.

(iv) The inner granular layer (0·36 mm.) is a fairly broad compact lamina of small rounded cells.

(v) The inner pyramidal or ganglionic layer (0·20 mm.) contains medium-sized polygonal and pyramidal cells. The largest cells of the visual cortex are the pyramidal cells of this layer; they represent the solitary cells of Meynert.

(vi) The fusiform layer (about 0·54 mm.) with medium-sized cells of different shapes (mostly rather small pyramids) is broad but ill-defined.

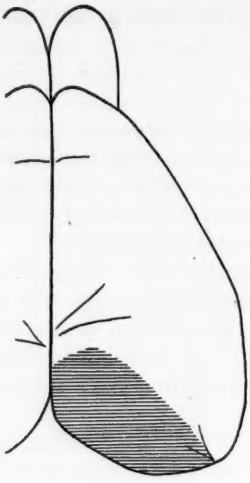

Fig. 2. A dorsal view of the hemisphere showing the extent of the area striata. Magnification $\times 2\frac{1}{2}$.

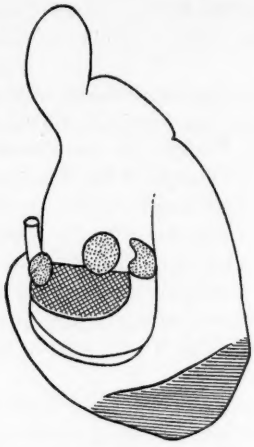

Fig. 3. Similar to Fig. 2, but showing the extent of the area striata on the medial surface of the hemisphere.

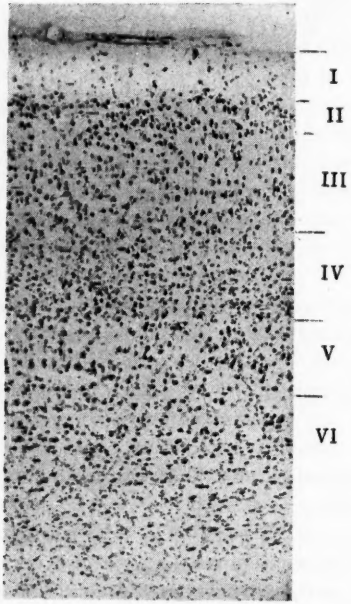

Fig. 4. Photograph of a section of the area striata. This is a paraffin section cut at 15 μ and stained with cresyl violet. Magnification $\times 45$.

Layer IV could not be subdivided into three parts as Brodmann (1909) has done in the case of another marsupial (kangaroo), but it consists of a single broad layer with the stria of Gennari coinciding with its outer part. Gray (1924) has reported a similar undivided layer IV in *Didelphys virginiana*, and Clark (1925) was unable to confirm Brodmann's findings in the kangaroo.

EXPERIMENTAL STUDY

The retinal projection

The following account of the retinal projection on the primary optic centres confirms and elaborates most of the findings in normal material. It is based on four unilateral eye enucleation experiments: two Marchi (P 28 and P 36) and two transneuronal degeneration specimens (P 43 and P 44). In P 43 the brain was stained with toluidin blue, in P 44 with Davenport's silver method.

One Marchi specimen (P 36) and one transneuronal specimen (P 43) will be reported in full.

P 36.

Operation (enucleation of right eye), 6 August 1939. The animal was killed on 28 August, and the brain treated by the Marchi method.

Optic nerve and chiasma (Fig. 5). Fairly dense Marchi degeneration is evenly distributed throughout the right optic nerve (Fig. 5 A); no degeneration is present in the left nerve. There is no evidence therefore for the presence of inter-retinal fibres.

In the chiasma (Fig. 5 B, C) the crossing of fibres and the relation of crossed to uncrossed fibres is well shown by this specimen. The uncrossed fibres are seen to maintain a ventrolateral position throughout the chiasma, but posteriorly (Fig. 5 C) a number of crossed fibres also come to occupy this position, scattered among the more numerous uncrossed fibres. Crossed fibres are found mainly in the dorsal and medial regions of the chiasma. The ratio of crossed to uncrossed fibres is approximately 3 : 1.

Fibres which probably belong to Gudden's commissure are found in the posterior part of the chiasma (Fig. 5 C). In the optic tracts these fibres pass in a dorsolateral direction with Meynert's fibres and come to occupy the deepest part of the tract where they are not mingled to any great extent with the retinal fibres. The position of the commissures of Gudden, Meynert and Ganser is shown in Fig. 5 D.

Optic tract. At the beginning of the tract (Fig. 5 D) the uncrossed fibres are mainly superficial with the crossed fibres deeply situated; when the upper border of the cerebral peduncle is reached intermingling has taken place and the Marchi granules are distributed evenly throughout each tract except for the deepest part where Meynert's and Gudden's fibres are present.

Following the homolateral tract, it is found that degenerated fibres are present only in its lateral division, and the great majority of these fibres enter

the dorsal nucleus of the lateral geniculate body. Some degenerated fibres appear to run past this nucleus, but they are so few in number that it is not possible to follow them with confidence to their destination. There is no definite evidence that uncrossed retinal fibres terminate anywhere but in the dorsal lateral geniculate nucleus, but this finding cannot be taken as excluding the possibility of some midbrain connexions.

In the heterolateral tract the degenerated fibres are more numerous and have a wider distribution, and can be followed into both its divisions. The granules present in the medial division are not numerous and for the most part

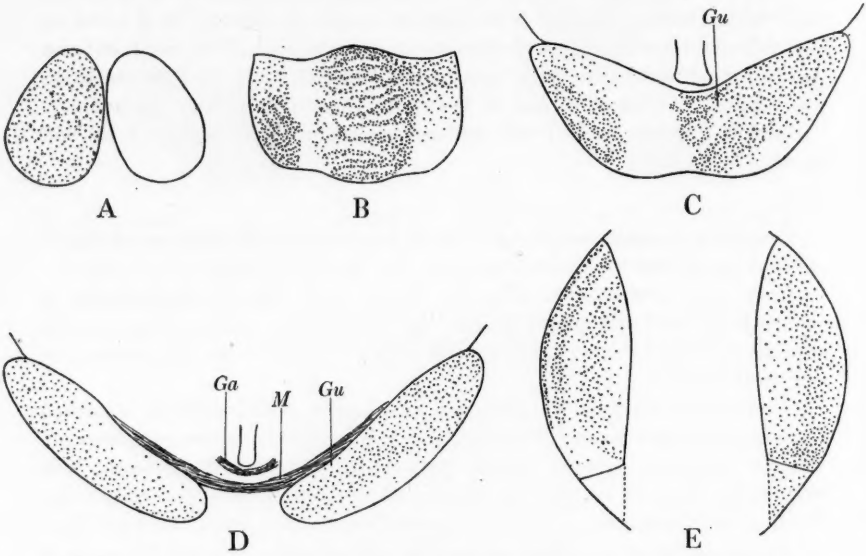

Fig. 5. Transverse sections showing the optic nerves (A), the chiasma (B and C), the optic tracts (D) and the lateral geniculate bodies (E) of P36. This specimen was treated by the Marchi method after enucleation of one eye. The stippling indicates the distribution of degenerated fibres on the homolateral (left) and heterolateral (right) sides in each drawing. *Ga*, Ganser's commissure; *Gu*, Gudden's commissure; *M*, Meynert's commissure.

cannot be followed beyond the ventral part of the dorsal nucleus of the lateral geniculate body. Degeneration in the lateral division is easily followed into the dorsal lateral geniculate nucleus and the superior colliculus of the midbrain; although the deeper degenerated fibres in this division pass through the large-celled nucleus of the optic tract and the superficial part of the pretectal nucleus, none of them appears to end there. Fine Marchi degeneration is present in the heterolateral ventral nucleus of the lateral geniculate body lying outside the fibre bundles which pass through it; this is taken as evidence that crossed retinal fibres (or their collateral branches) end in this nucleus.

On the heterolateral side degeneration is found in both the tractus peduncularis transversus and the anterior accessory optic tract. These two tracts join together a little anterior to the nucleus opticus tegmenti and degeneration granules can be followed into this nucleus. No crossed or uncrossed retinal fibres could be traced to any other parts of the brain.

Lateral geniculate body. In the dorsal lateral geniculate nucleus a definite lamination pattern has been seen already in normal material and the main interest will centre on the relation of the terminating optic fibres to this lamination. Marchi material indicates that a definite relation does exist (Fig. 5 E). On the ipsilateral side Marchi granules are found throughout the nucleus except in its ventral quarter: the granules, however, are not evenly distributed but are arranged in three zones. A narrow outer zone of degeneration coincides with lamina 1; most of the granules found here are large, and it may be that they are due to degeneration in fibres of passage, rather than in fibres which are terminating in this layer. Corresponding in position to lamina 2 is another zone of degeneration, while medial to this is a fairly broad layer (in the position of lamina 3) practically free from degeneration. The third zone of degeneration is broad and corresponds in position to laminae 4 and 5: few granules are present in the medial non-laminated portion. These zones of Marchi degeneration are constant in relative position throughout the nucleus. Several Marchi sections were counterstained with toluidin blue and, although the stain did not take well, it was good enough to confirm the fact that the three zones of degeneration coincide with laminae 1, 2 and 4–5, and that the region of scanty degeneration in the ventral quarter of the nucleus corresponds fairly accurately with the region in which laminae 1, 2 and 3 are intermingled. No Marchi degeneration is present in the ipsilateral ventral lateral geniculate nucleus.

On the contralateral side degeneration is found throughout the dorsal nucleus, with the granules more plentiful in the ventral quarter and the peripheral half of the nucleus (Fig. 5 E). Zones of degeneration are not present although the granules are more numerous and more densely packed in the region of lamina 3. Fine Marchi degeneration is present in the substance of the contralateral ventral nucleus.

Marchi granules are present in the contralateral superior colliculus. They are most abundant in the stratum opticum but are present to a small extent in the stratum griseum superficiale (but not in the stratum zonale). Granules are more numerous in the lateral part of the laminae (where the optic fibres enter), and in a bundle of fibres lying at the medial border of the stratum opticum and running posteriorly; these fibres also terminate in the superior colliculus, for the bundle gradually dwindles and is lost before the posterior border of the superior colliculus is reached.

The findings in P 28 (Marchi) were similar to those reported above in P 36. P 44 (Davenport silver) was useful in confirming the Marchi results with regard to the relation of crossed to uncrossed fibres in the chiasma and tracts. Also in P 44 no normal fibres could be found in thin transverse or longitudinal

sections of the degenerated optic nerve. This seems to exclude the possibility that efferent fibres are present in the optic nerves.

P 43.

Operation 29 November 1939. Right eye enucleated. Killed 11 March 1940 (survival period, 100 days); sections stained with toluidin blue.

Apart from the expected increase in the number of neuroglial cells found in each optic tract the only positive findings in this specimen concern the dorsal nuclei of the lateral geniculate bodies. No changes were observed in any other nuclei. The cutting of the optic nerve causes cells in both dorsal nuclei to become shrunken, to stain poorly, and to show various stages of chromatolysis; it is probable that no cells are lost completely. In the homolateral nucleus chiefly alternate laminae are affected (viz. laminae 2 and 4); in the heterolateral nucleus the other set of alternate laminae shows degenerative changes (viz. laminae 1 and 3).

Homolateral dorsal nucleus. In lamina 1 there are a few cells present which show chromatolytic changes but the majority are normal. In lamina 2 most cells show the characteristic degenerative changes; a few apparently normal cells are present throughout, but the ill-defined limits of lamina 2 might account for this finding. Lamina 3 appears normal with a fairly conspicuous row of cells along its inner border. Lamina 4, which is narrow but well defined throughout its extent, is practically completely degenerated, and very few normal-looking cells can be found in it. In lamina 5 and in the non-laminated portion medial to it both normal and degenerated cells are present; in lamina 5 most of the cells appear to show degenerative changes, whereas in the non-laminated portion the majority are normal. However, due to the fact that a definite fifth layer can be seen only in a few sections passing through the centre of the dorsal nucleus, the observations on the degeneration changes in this lamina cannot be regarded as conclusive. In the ventral quarter of the nucleus where laminae 1, 2 and 3 are intermingled, all the cells appear normal.

Heterolateral dorsal nucleus. In lamina 1 the majority of the cells present show degenerative changes, but some normal cells are present. Lamina 2 is apparently normal, but in 3 all cells are degenerate. In laminae 4 and 5 only normal cells can be seen, while in the non-laminated portion a few cells show chromatolytic changes, but normal cells are in the majority. In the ventral quarter of the nucleus practically all the cells show degenerative changes.

In P 44 (Davenport silver) very little change was noticed in the neuropile of any optic centres, and the specimen could not be used to confirm the findings in P 43. A similar negative result has been reported by Bodian (1935) in the opossum.

The positive results from the eye enucleation experiments may be summarized as follows:

The fibres in the optic nerves are all afferent, and at the optic chiasma a partial crossing of fibres occurs. About one-quarter of the fibres present in each nerve do not cross; they occupy a ventrolateral position throughout the

chiasma and a superficial position in the beginning of the tract. Crossed fibres occupy the greater part of the rest of the chiasma and a deeper position in the beginning of the tract.

In the tract the fibres from the two retinae do not remain separate for long but soon become intermingled. Most fibres enter the lateral division of the tract, but a few crossed fibres enter the medial division. In spite of their intermingling, crossed and uncrossed fibres end, for the most part, in different regions; even when they go to the same nucleus the two sets of fibres separate out to end in different parts of that nucleus.

The dorsal nucleus of the lateral geniculate body is the only nucleus which undoubtedly receives fibres from both retinae. The termination of these fibres bears a definite relation to the lamination, and for the most part the two sets of fibres terminate in two sets of alternate laminae. Crossed fibres end chiefly in laminae 1 and 3 and in the ventral quarter of the nucleus where laminae 1, 2 and 3 are fused together. There is evidence that a few crossed fibres also end in relation to cells in lamina 2 and the non-laminated portion. Uncrossed fibres end chiefly in laminae 2 and 4 (and possibly 5); a few also end in lamina 1 and the non-laminated portion. No uncrossed fibres end in the ventral quarter of the dorsal nucleus.

Crossed fibres appear to end also in the ventral nucleus of the lateral geniculate body, and throughout the stratum opticum and stratum griseum superficiale of the superior colliculus and in the nucleus opticus tegmenti. They may have connexions in the nucleus lateralis posterior of the thalamus and in the pretectal nucleus, although there is no positive evidence for this. No evidence could be found that uncrossed fibres end in any of these structures, although some do appear to pass beyond the dorsal nucleus of the lateral geniculate body. These may go to the midbrain as in the opossum (Bodian, 1935), but were too few to be traced with certainty in the phalanger.

The geniculo-striate projection. In the phalanger (as in the other mammals studied) a lesion destroying some of the visual cortex produces a localized retrograde cell degeneration in the homolateral dorsal geniculate nucleus. The degeneration consists in the death and complete destruction of cells by neuronophagia, and is accompanied by neuroglial proliferation which clearly demarcates the degenerated zone. A few normal cells can usually be found in a degenerated zone, particularly towards its periphery.

In each of six animals a single lesion was made involving the area striata, or the area striata together with more or less of the adjacent cortical areas. The lesions differed in size and position in each specimen. In each, a definite zone of degeneration could be fairly accurately localized in the homolateral dorsal geniculate nucleus; moreover, the position and size of this zone varied with the position and size of the cortical lesion. It was possible, therefore, by combining the results of all experiments to localize with fair accuracy the projection of the lateral geniculate body on the striate area. To demonstrate this projection four experiments have been selected for description.

P 50 (Fig. 6).

Operation 15 March 1940. A small lesion made in the posterior part of the visual cortex with a sharp scoop. Killed 4 May 1940 (survival period, 50 days); sections stained with toluidin blue.

The small lesion present in this specimen does not extend into the subcortical white matter to any appreciable extent, but it does extend a little into the peristriate cortex.

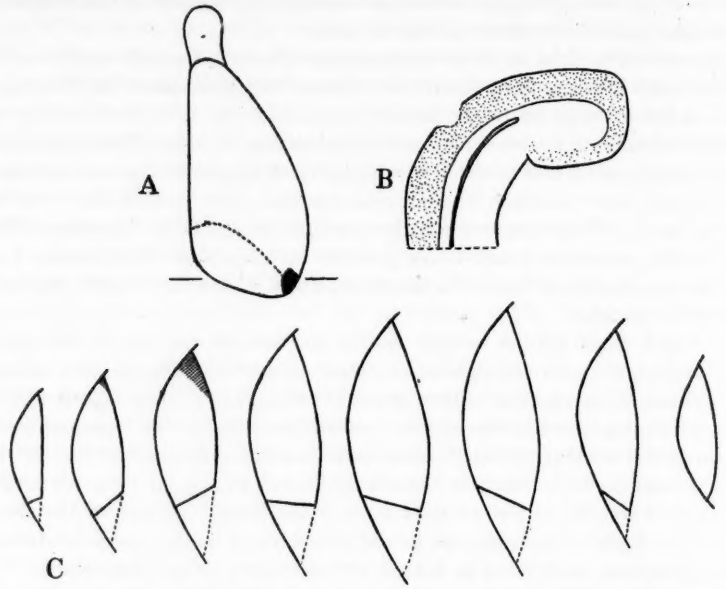

Fig. 6. P 50. The drawing A shows the position of the lesion on the hemisphere and its relationship to the area striata. B is a section showing the depth of the lesion. C is a series of transverse sections through the lateral geniculate body, equally spaced from the anterior end on the left to the posterior on the right. Degeneration in the lateral geniculate body is represented by oblique shading.

The area of degeneration in the dorsal lateral geniculate nucleus is small and not as well defined as in the case of larger cortical lesions. It is present in the anterior and dorsal part of the nucleus and extends through twelve sections (40 μ) only. The degeneration extended slightly into the nucleus lateralis posterior of the thalamus, probably the result of injury to the peristriate area.

P 51 (Fig. 7).

Operation 20 March 1940; a lesion made in the anterior part of the striate area with a small scoop. Killed 4 May 1940 (survival period, 45 days); sections stained with toluidin blue.

The slightly larger lesion in this specimen does not extend outside the striate area and has not appreciably affected the subcortical white matter. The extreme anterior part of the area striata has been destroyed except for a small part anteromedial to the lesion. The resulting degeneration is found in the posterior and ventral part of the homolateral dorsal geniculate nucleus, but is not complete. A small region remains in the posteroventral part of the nucleus in which normal cells predominate, and it is probable that fibres from this region project on to the small area of intact visual cortex anteromedial to the lesion. No degeneration was found in any other nuclei.

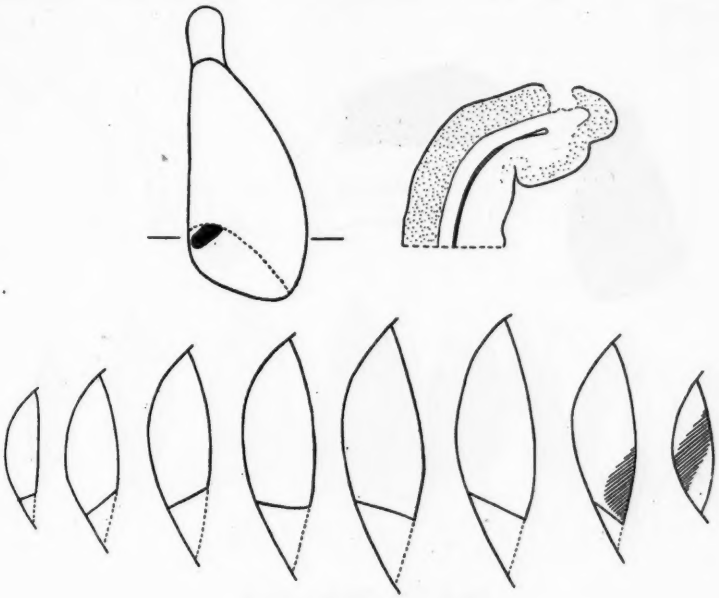

Fig. 7. P51. Similar to Fig. 6.

P 16 (Fig. 8).

Operation 22 August 1939; an extensive lesion made in the occipital and parietal regions of the hemisphere with the diathermy. Killed 15 November 1939 (survival period, 86 days); sections stained with toluidin blue.

In this specimen the main lesion is outside the area striata but it has been included because all fibres from that area have been cut off except those from its most posterior and lateral part. The homolateral dorsal geniculate nucleus is almost completely degenerated, but a zone of normal cells is present in its anterodorsal part which presumably projects on to the intact posterolateral part of the area striata. Extensive degeneration is present in other thalamic nuclei; this will be reported in future communications from this laboratory.

P 15 (Fig. 9).

Operation 18 August 1939; a fairly large lesion made in the middle of the striata area with the diathermy. Killed 16 November 1939 (survival period, 91 days); sections stained with toluidin blue.

The lesion, which extends into the subcortical white matter, is so situated that it leaves a small anterior and a large posterior part of the area striata intact. On account of the subcortical involvement, all fibres running to the cortex posteromedial to the lesion have been divided. Corresponding to the lesion a large part of the dorsal geniculate nucleus is degenerated. There is,

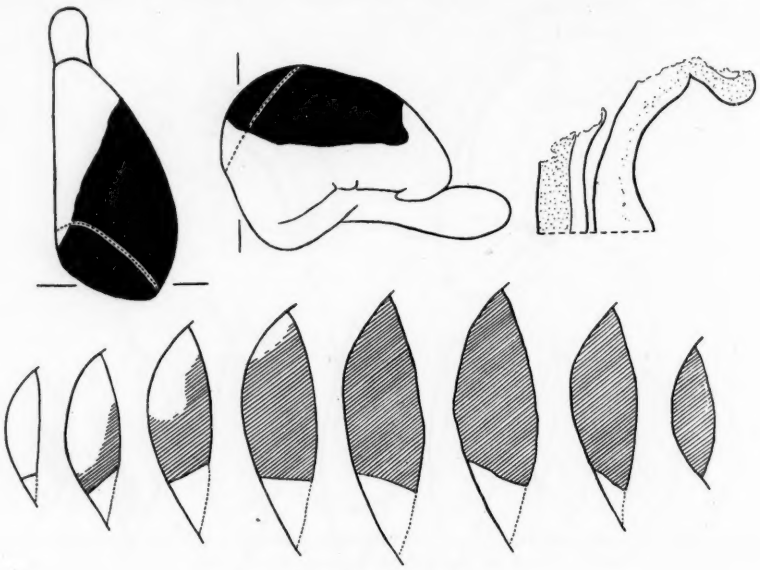

Fig. 8. P16. Similar to Fig. 6.

however, a large anterior and a small posterior part of the nucleus which is undegenerated, these undegenerated parts corresponding to the posterior and anterior intact parts of the area striata respectively.

Other specimens with lesions affecting the area striata gave results similar to those reported above, and the following conclusions may be reached with regard to the localization of the geniculo-striate projection.

Although the number of experiments is small it appears that lesions confined to the area striata lead to degeneration in the homolateral dorsal geniculate nucleus only; it seems certain, also, that at least the great majority of projection fibres from this nucleus to the cortex go to the area striata. It is possible that some fibres go to the peristriate area also, but as lesions made in this area affected the area striata also this point remains undecided.

There is present a reversed representation of the dorsal geniculate nucleus in the striate area, so that fibres from the anterior pole of the nucleus are projected on to the posterior part of the area striata and vice versa. It is also probable that the dorsal part of the nucleus is represented in the lateral part of the striate area (see P 50, Fig. 6 and P 16, Fig. 8), and although direct evidence is lacking, it can be inferred that fibres from the ventral part of the nucleus end in the medial part of the striate area.

A composite diagram showing the projection of the dorsal nucleus of the lateral geniculate body on the striate area was not prepared, for no evidence

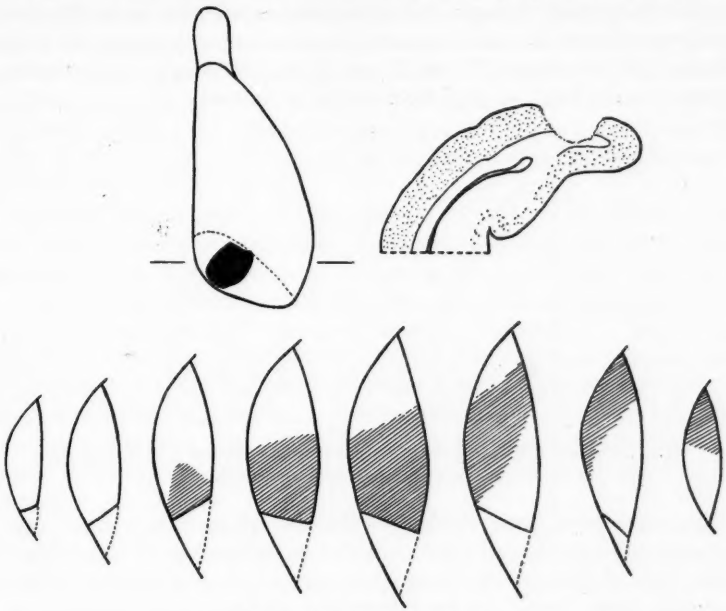

Fig. 9. P 15. Similar to Fig. 6.

was obtained which would make the localization of this projection differ essentially from that in the American opossum (Bodian, 1935, Figs. 9, 10), and in any case more experiments would be necessary for the preparation of a detailed projection map. The character of the degeneration and its degree of localization is very similar to that in the American opossum (Bodian, 1935), and in general no fundamental difference has been found between the geniculostriate projection in the phalanger and in any other mammal investigated.

The corticofugal fibres from the area striata

The following account is based on the findings in two Marchi specimens, P 42 and P 38 (Fig. 10). Although the lesions differed in position in the two specimens, in each case it was of moderate size and confined to the visual cortex

and the underlying fibres. Both experiments gave similar results and will therefore be reported together.

Degenerated fibres can be followed in the deepest stratum of the white matter of the hemisphere (next to the ependyma of the ventricle) to the posterior part of the internal capsule. From the internal capsule some enter the external medullary lamina of the thalamus, others enter the cerebral peduncle and can be followed posteriorly near its lateral border. As the peduncle enters the midbrain the degenerated fibres in it turn dorsally to join the posterior part of the optic tract; they can be followed in both deep and superficial divisions of the tract, through or over the lateral geniculate body, the nucleus lateralis posterior of the thalamus and the pretectal nucleus into the stratum opticum of the superior colliculus. Some of these fibres may end in the lateral geniculate body, but if so they are very few in number.

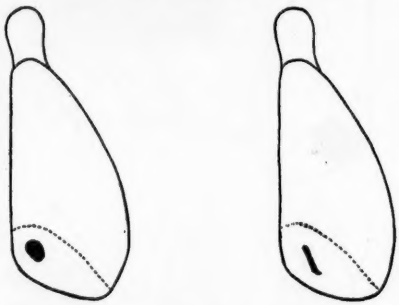

Fig. 10. P 42 and P 38. The lesions in the area striata are shown; these specimens were treated by the Marchi method.

The degenerated fibres which enter the external medullary lamina (via the intermediate thalamic radiations) can also be followed posteriorly. They run in the part of the external medullary lamina which separates the lateral geniculate body (dorsal nucleus) from the lateral nuclear complex of the thalamus, and in the lamina separating the dorsal and ventral nuclei of the lateral geniculate body (Fig. 1). They appear to end only in the dorsal nucleus of the lateral geniculate body, and chiefly in its ventral half.

The corticofugal fibres from the striate area are therefore of two main kinds: corticothalamic, running through the thalamic radiation and external medullary lamina to the lateral geniculate body; and corticomesencephalic, running through the cerebral peduncle to the superior colliculus. A similar subdivision in the corticofugal fibres from other areas of cortex can be made, but in most cases the fibres which enter the cerebral peduncle descend to lower levels than the superior colliculus of the midbrain. Apparently no regions posterior to the superior colliculi receive fibres from the area striata.

By comparing the above specimens with the Marchi material prepared after eye enucleation, it can be seen that the deep or medial division of the

optic tract is composed mainly of fibres of cortical origin; the lateral (superficial) division is composed mainly of fibres of retinal origin, although neither division contains exclusively one type of fibre.

DISCUSSION

In general, the visual system of the phalanger shows an advanced state of development as compared with most primitive mammals (Goldby, 1940). This is indicated by the large size and intrinsic differentiation of the lateral geniculate body, and by the number of uncrossed fibres in the optic tracts. So far as the position of the eyes is concerned there does not seem to be much difference between the phalanger and the opossum; in the latter Bodian (1937) has found that the angle between the optical axes is 60°. At the same time the proportion of uncrossed fibres in the optic tracts appears to be larger in the phalanger than in the opossum, and this may indicate a higher degree of development of binocular vision.

In the phalanger crossed retinal fibres have been shown to end in both nuclei of the lateral geniculate body, the superior colliculus and in the nucleus opticus tegmenti. These results are in general agreement with those reported for other mammals, but a number of points need further discussion.

Most investigators have described uncrossed as well as crossed retinal connexions with the superior colliculus, e.g. in the opossum (Bodian, 1937), the rat (Lashley, 1934), the rabbit (Brouwer, 1927; Overbosch, 1926), the cat (Barris *et al.* 1935) and the monkey (Brouwer & Zeeman, 1926). Such midbrain connexions have not been demonstrated in the phalanger, although it is possible that a small number of uncrossed retinal fibres do reach the superior colliculus.

On the evidence of Marchi material, retinal connexions to the heterolateral ventral geniculate nucleus appear to be present in the phalanger; fine Marchi granules lying outside the bundles of fibres traversing this nucleus suggest such an interpretation. This, while agreeing with the results obtained in some mammals, is contrary to those in a number of others. It would appear that the question of retinal connexions to the ventral nucleus of the lateral geniculate body is doubtful and will need further investigation.

Both the nucleus lateralis posterior of the thalamus and the pretectal nucleus (including the large-celled nucleus of the optic tract) are traversed by fibres, some at least of which are of retinal origin. No evidence was found that any of them end in these regions; they appear all to be fibres of passage, going to the superior colliculus. Similar findings have been reported in other animals, notably by Brouwer (1927) in the rabbit, cat and monkey. Such negative evidence, particularly when based on Marchi material, cannot exclude entirely the possibility of retinal connexions with the thalamus or pretectal nucleus; it is fair to conclude, however, that such connexions, if present at all, must be very scanty compared with the lateral geniculate or midbrain connexions.

The lamination of the dorsal nucleus of the lateral geniculate body is the most striking feature of the optic system in the phalanger. In many mammals there is no lamination, e.g. in rodents (Gurdjian, 1927), monotremes (Hines, 1929) and in the other marsupial (the opossum) which has been described (Tsai, 1925). The maximum number of laminae described in any mammal is six (as in the higher primates), and there are many stages between the non-laminated and this highly differentiated form. Thus some animals such as the porpoise and the seal (Putnam, 1926) show incipient lamination, with a single convex superficial lamina of large cells; in *Tupaia* (Clark, 1929) there are several laminae parallel to the surface, and the phalanger is similar. In many of the more advanced mammals the laminae tend to take on complex forms, as in the lateral geniculate body of the Anthropoidea (Clark, 1934), or the S-shaped arrangement found in Carnivora (Thuma, 1928; Rioch, 1929).

In the phalanger four, and possibly five, laminae have appeared lateral to a non-laminated portion. Their general form is relatively simple; they are parallel to the surface and partially encircle the non-laminated portion, with most of the optic tract fibres entering along the lateral convexity, and the optic radiation leaving from the medial concave side. In the shape of the laminae the phalanger may show an advance on the vertically laminated dorsal geniculate nucleus of *Tupaia*, resembling the lemurs (e.g. *Microcebus*) and tending towards the inverted type of lateral geniculate body.

The type of cell found in the lateral geniculate body appears to vary considerably in different mammals; and it is difficult to institute precise comparisons; however, in most groups (e.g. Primates) the outer one or two laminae consist of large cells. The single superficial lamina in the porpoise and seal consists of large cells. This feature is also characteristic of the phalanger; it is found in the cat, but not in the dog.

It has been shown by the transneuronal degeneration technique that in several forms, e.g. man (Clark, 1932; Mackenzie, 1934; and others), monkey (Minkowski, 1920; Clark, 1932; Clark & Penman, 1934), cat (Minkowski, 1920*a*), the termination of retinal fibres is definitely related to the lamination pattern of the dorsal lateral geniculate nucleus, so that crossed fibres end in one set of laminae and uncrossed fibres in the alternate set. This is also true of the phalanger in which crossed fibres end chiefly in laminae 1 and 3, and uncrossed chiefly in laminae 2 and 4, a partial confirmation of this finding being obtained from Marchi material. It is probable that lamina 3 receives only crossed fibres, and lamina 4 only uncrossed fibres, since after enucleation of an eye either all or none of the cells in these laminae show degenerative changes, according to the side which is being examined. In the other laminae there is some overlap of crossed and uncrossed fibres, since they are neither completely degenerated nor completely normal in either right or left lateral geniculate body after enucleation of one eye. It appears that in the higher primates, e.g. man (Clark, 1932), the segregation of crossed from uncrossed fibres is much more complete.

The lateral geniculate body of the phalanger therefore shows a stage intermediate between the fully laminated condition seen in Carnivora and in the higher primates, and the non-laminated condition in such animals as the rat and the opossum. The degree of lamination is probably related to the degree to which binocular vision has been developed, a suggestion which is strengthened by comparison of the region of the lateral geniculate body which receives only crossed fibres in these animals, and is presumably concerned with monocular vision. In the rat (Lashley, 1934), the rabbit (Brouwer, 1927; Overbosch, 1926) and the opossum (Bodian, 1937), the ventral third or more of the non-laminated dorsal nucleus receives only crossed fibres; the corresponding region in the laminated dorsal nucleus of primates is very small, partly masked by the lamination pattern. It was identified by Brouwer in the monkey (1927). Between these extremes lies the lateral geniculate body of the phalanger. Throughout its dorsal nucleus, laminae 1, 2 and 3 are fused ventrally, the area of fusion occupying about one-quarter of the whole cross-sectional area of the nucleus; both transneuronal and Marchi degeneration specimens indicate that this region receives only crossed retinal fibres, and is presumably associated with monocular vision. It is noteworthy that the relative size of this region is less than in the rat or the opossum, but greater than in the monkey.

So far as they go, the experiments bearing on the geniculo-striate projection system show an essential similarity to that system in other mammals. The experiments are less numerous and less precise than those of Bodian (1937), so that detailed differences may have escaped detection. It is also doubtful if the area striata differs significantly in its histology from the same area in other primitive mammals. Although it is noticeable that lamina IV contains fewer cells along its outer border, there is no suggestion of a subdivision of this lamina like that found in *Tarsius* and the Anthropoidea, and less clearly in *Tupaia* and the Lemuroidea (Clark, 1925). It resembles very closely the area striata of the rabbit, described and illustrated by Rose (1931) and also that of the opossum (Gray, 1924). Detailed comparisons would no doubt show differences, but to judge their significance a first-hand knowledge of the area striata in a series of mammals is necessary. In comparing with published accounts it is difficult or impossible to allow adequately for differences in the technique of preparing the sections; such differences may have considerable influence on the appearance of cortical areas.

SUMMARY

The normal anatomy and histology of the optic chiasma, optic tracts, lateral geniculate body, superior colliculus and area striata of the cerebral cortex have been studied by means of serial sections stained by the Weigert method (Weil's modification) and by toluidin blue. These structures have been shown to be essentially similar to those in other primitive mammals (marsupial or placental) except for the marked lamination of the lateral geniculate body.

Their connexions have been studied by the use of the Marchi, retrograde and transneuronal degeneration techniques, following enucleation of an eye, or the placing of lesions in the area striata. The experimental results are summarized as follows:

(i) All fibres of the optic nerve are myelinated, all degenerate after section of the nerve, and about 75 % of them cross in the optic chiasma. The 25 % which remain uncrossed lie ventrolaterally in the chiasma, and superficially in the beginning of the optic tract; as the tract passes dorsally they become mixed with crossed fibres.

(ii) Crossed retinal fibres can be traced to the dorsal and ventral nuclei of the lateral geniculate body, to the superior colliculus, and to the nucleus opticus tegmenti via the anterior accessory optic tract and the transverse peduncular tract. Uncrossed retinal fibres can be traced only to the lateral geniculate body (dorsal nucleus), but the possibility that a few go to the superior colliculus cannot be excluded. No evidence was found for retinal connexions with the nucleus lateralis posterior of the thalamus or the pretectal nucleus; if such connexions are present they must be scanty.

(iii) In the lateral geniculate body (dorsal nucleus) crossed and uncrossed fibres end chiefly in alternate laminae; the ventral quarter of this nucleus is poorly laminated and receives only crossed fibres.

(iv) Corticopetal fibres from the lateral geniculate body end mainly (probably exclusively) in the area striata, where there is a reversed representation of the parts of the lateral geniculate body similar to that described in the opossum (Bodian, 1935) and other mammals. Corticofugal fibres from the area striata can be traced to the lateral geniculate body (dorsal nucleus) and to the superior colliculus of the midbrain, but to no other parts of the nervous system.

My thanks are due to Prof. F. Goldby of the University of Adelaide, without whose advice and criticism this work could not have been carried out. Acknowledgement must also be made for the help of the technician, Mr R. Jarrett, who prepared most of the sections.

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THE SENSORY COMPONENT OF THE HYPOGLOSSAL NERVE IN THE RABBIT

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In 1937 I published a short note reporting that Tarkhan's (1936) description of a collection of sensory cells on the course of the hypoglossal nerve in the rabbit could not be confirmed. Tarkhan stated that these nerve cells formed a small ganglion on the trunk of the nerve in the neighbourhood of the origin of its descendens branch, and he considered them to be the cell bodies of the proprioceptive fibres from the tongue musculature. I was also unable to verify Langworthy's statement (1924) that ganglia of the Froriep type are 'fairly constantly' to be found in the rabbit. Since the publication of this note I have added considerably to my material, especially in the embryonic and late foetal stages and, as the following account will indicate, no evidence for the occurrence, even occasionally, of sensory cells on the course of the hypoglossal nerve has been found, and in none of the large number of embryos which have been examined by dissection and in serial section has a hypoglossal dorsal root ganglion of the Froriep type been identified. This negative finding is in agreement with the results of Beck (1895) and of Froriep & Beck (1895) who were unable to identify a dorsal root or sensory ganglion connected with the hypoglossal nerve in the rabbit. Indeed, of all the rodents examined by them such a ganglion was only found once, in a guinea-pig, and was not verified by histological examination.

Carleton (1938) was also unable to find sensory cells on the extra-cranial part of the rabbit's hypoglossal and, in agreement with my results, could not identify muscle spindles in the lingual musculature of the rabbit and other mammals. Her observations suggested that proprioceptive innervation may not exist in the tongue musculature, but if it does exist the afferent fibres must pass back in the hypoglossal nerve as there is complete degeneration of nerve endings in direct relation to muscle fibres after hypoglossal section. Weddell and co-workers (1940), using a modified and much improved intra-vitam staining technique, were also unable to identify muscle spindles in the intrinsic or extrinsic muscles of the tongue of rat and rabbit (one) material. They described, however, sensory endings of a tension-recording type in the genioglossus muscle near its origin. Although the type of ending found by them does not correspond in structure to the neuro-tendinous endings of Golgi, resembling rather the 'ball-like' nerve terminations described by Stoehr (1932) in the pia-mater, they regarded it as subserving similar functions to the Golgi endings, responding to active contraction or passive stretch. Presumably, if

such endings are concerned in the lingual proprioceptive mechanism, the fibres arising from the endings pass for part of their course in the trunk of the hypoglossal nerve, but their course, and the situation of their cell bodies, were not determined. The hypoglossal nerve, which was only examined in its peripheral portion, was never found to have sensory cells on its course.

In addition to the interest of the question of the presence or absence of sensory cells on the course of the hypoglossal nerve for the problem of the proprioceptive innervation of the tongue, there is the morphological issue of Froriep's ganglia. From time to time these have been reported in a number of different mammals, mostly, however, from the findings of gross dissections, and there has been no serious histological study of them. They appear to be present, more or less constantly, in Artiodactyla, frequently in Carnivora and rarely in man. Rodents, as has been stated above, were found by Froriep & Beck to have no hypoglossal dorsal root ganglia, the only exception being one specimen of *Cavia*. The differing frequency of their occurrence in different mammals and the absence of reliable histological data on their cell content and fibre connexions have left the nature and functions of these ganglia very vague. It has, therefore, been made part of the study of the rabbit hypoglossal nerve to investigate its developmental history from the point of view particularly of the presence or absence of ganglia of the Froriep variety.

MATERIAL AND METHODS

A number of dissections were made of the hypoglossal nerve and its connexions in fully grown rabbits and in embryos and full-term foetuses. Four hypoglossal nerves were dissected in the manner recommended by O'Connell (1936) in an attempt to determine the nature of its intra-neural plexus.

Rabbit embryos from the 4 mm. stage to full term have been available in serial and transverse sections. These embryos are stained with haematoxylin and eosin for the most part, but a number (5, 8, 10, 13, 17·5, 22, 36, and 56 mm. stages and four full-term foetuses) are stained with the Ranson pyridine silver or the de Castro (1925) modification of the Cajal techniques. The earlier embryos are represented by series cut in sagittal and frontal as well as transverse planes.

OBSERVATIONS

Anatomy of the hypoglossal nerve in the rabbit

The hypoglossal nerve in the rabbit arises by numerous rootlets from a longitudinal groove on the ventral surface of the medulla lateral to the poorly defined pyramidal elevation. In none of the large number of rabbit brains examined (over forty) has a dorsal hypoglossal root or a dorsal root ganglion been found. The rootlets of origin of the nerve unite, near the occipital bone, to form a cranial and a caudal hypoglossal root. These roots leave the skull

through separate openings (foramina condyloidea superius et inferius) in the occipital bone near the margin of the foramen magnum. They join almost immediately on leaving the skull and the resulting single hypoglossal nerve trunk lies posterior to the vagus nerve and the upper part of its ganglion nodosum. The hypoglossal trunk then passes laterally and forwards, swinging round the ganglion nodosum in the angle between it and the spinal portion of the accessory nerve which has left the vagus complex at the upper end of the ganglion. In this situation the hypoglossal nerve is closely adherent to the ganglion nodosum and, on dissection, the two structures can only be separated with some difficulty. This adherence is due, as my sections show (see Pl. 1, fig. 4), to the partial fusion of the fibrous tunics of the nerves and not to any nervous connexion or exchange of nerve fibres between them.

Below the point of formation of the single hypoglossal trunk a bundle of nerve fibres can sometimes be found joining the nerve and it is derived from the anterior primary division of the first cervical nerve. In most dissections, however, this communication is established by way of the descendens hypoglossi. Occasionally communications were found between the trunk of the hypoglossal nerve and the superior cervical ganglion of the sympathetic trunk. These sympathetic communications were inconstant both in size and position, but serial sections of a full-term foetus showed that the connexions were composed of true nerve fibres (see Pl. 2, fig. 5). In one dissection there was a large communication between the hypoglossal trunk and the glossopharyngeal nerve below its ganglion nodosum, but although this condition was carefully looked for in subsequent dissections it was not seen again. In no dissection was any evidence found for the existence on the trunk of the hypoglossal nerve of a ganglion and, as has already been stated, no trace of a ganglion could be located in connexion with its intracranial rootlets. The further course of the nerve to the tongue shows no noteworthy features. In the tongue itself there is some plexus formation between it and the terminal branches of the lingual nerve.

In those hypoglossal nerves which were dissected in the manner recommended by O'Connell (1936), an intraneural plexus was found to be present. The plexus formation, however, is not as complicated as O'Connell has shown it to be in many human nerves which contain both afferent and efferent fibres and, in particular, the finer component of the plexus was not found. As far as the results of dissections by this method are of significance, therefore, they do not suggest that the hypoglossal nerve is a mixed nerve, for it is the finer plexus which O'Connell has shown to be present in plurifunctional nerves. On the other hand, he thought that the coarser interfascicular communications may be related to the embryological history of the muscles innervated by the nerve in which they are to be found. This may be the explanation of the plexus which he found in the human hypoglossal nerve, and which can be found in the rabbit hypoglossal nerve, for both the tongue muscles and the hypoglossal nerve are plurisegmental in origin.

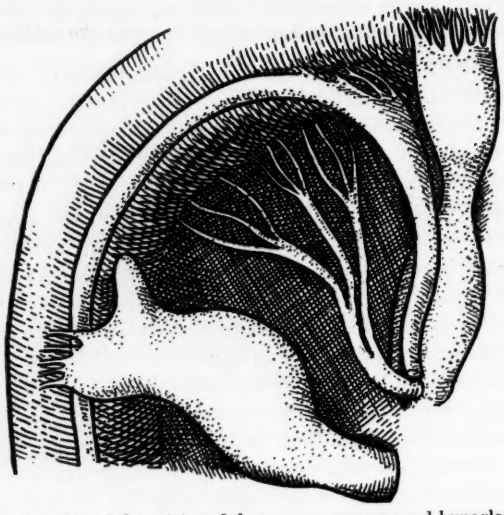
Development of the hypoglossal nerve in the rabbit

Kimmel (1940) has recently given a comprehensive account of the development and differentiation of the bulbar motor nuclei and of the associated root fibres in the rabbit. In general my results correspond closely with his, but he has not referred to the question of Froriep's ganglion or of a sensory component in the hypoglossal nerve.

In the 5 mm. embryo (11 days, 8 hr. post coitum) the hypoglossal nerve cells are situated in the posterior rhombencephalic portion of that common cell column which lies in the mantle zone of the basal lamina and from which all the efferent cranial nerves take their origin. Kimmel restricts the hypoglossal cells to the caudalmost neuromere (sixth rhombic groove of Streeter), but my material, although available in sagittal as well as transverse sections, will not allow of such a restriction as the cells giving rise to hypoglossal fibres overlap anteriorly to some extent the preceding neuromere and posteriorly are continuous, beyond the caudal limit of the sixth neuromere, with the better differentiated efferent cell column of the spinal cord. The cells giving origin to the hypoglossal fibres are intermingled with the efferent vago-accessory cells and, as described by Kimmel, but not in agreement with Windle's (1932) description in the cat embryo, the hypoglossal cells are for the most part situated medially and the vago-accessory cells laterally. There is, however, considerable intermingling of these cells at this stage. The fibres arising from the hypoglossal cells course ventrally and slightly laterally through the narrow marginal zone of the medulla and emerge on its ventro-lateral aspect in small bundles. The latter gradually converge to form two roots of which the posterior tends to remain as two secondary roots (see Text-fig. 1) which fuse together before joining the anterior root to form the hypoglossal nerve trunk.

In these 5 mm. embryos there is no appearance even suggesting a sensory root to the hypoglossal nerve. There are cells of neural crest origin which will develop into sensory cells of the vago-accessory complex dorsal to and on a level with the hypoglossal rootlets, but these cells are still ill-differentiated and have no peripheral processes. The first cervical ganglion is further advanced in its development and has a small projection forwards, but this projection has no fibres passing other than into the main mass of the ganglion behind.

In embryos of 7·5–8 mm. length (12 days, 12 hr. post coitum) the hypoglossal nerve cells show some sign of segregation from the continuous efferent column, but on the peripheral portions of the differentiating nucleus there is still considerable overlap of, laterally, hypoglossal and vago-accessory cells and, caudally, hypoglossal and anterior spinal motor nerve cells. The hypoglossal cells have increased in number, or, at least, more of them have now developed peripheral axones, so that the number of fasciculi of fibres has increased and the fasciculi themselves are stouter and stain more intensely. As in the earlier stages, the fasciculi leave the ventro-lateral part of the medulla (see Text-fig. 2) to form the trunk of the nerve. There is no dorsal

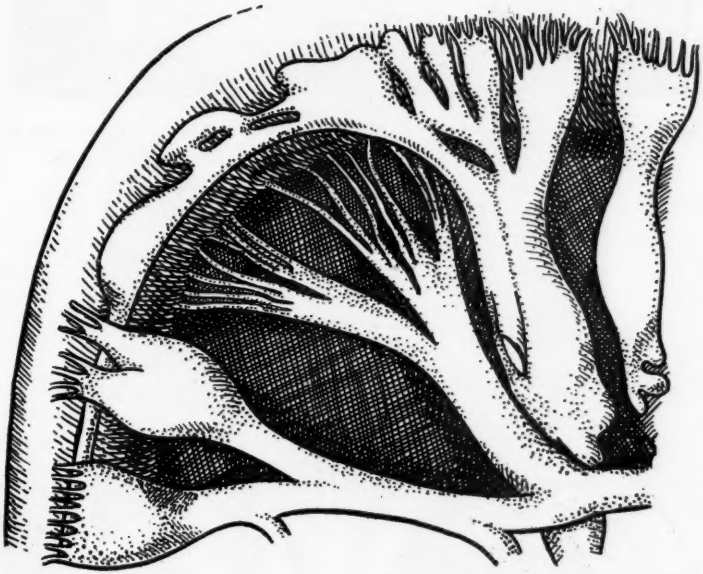


Text-fig. 1. Reconstruction of the origins of the vagus, accessory and hypoglossal nerves and of the first cervical dorsal root ganglion in a 5 mm. rabbit embryo.

Text-fig. 2. Sagittal section of the hypoglossal and first cervical rootlets in a 7·5 mm. rabbit embryo. Ranson pyridine silver. × 120.

root and no dorsal root ganglion. The vago-accessory sensory cells are more advanced in their differentiation but neither they nor the large first cervical dorsal root ganglion send any fibres to hypoglossal rootlets (see Text-fig. 3).

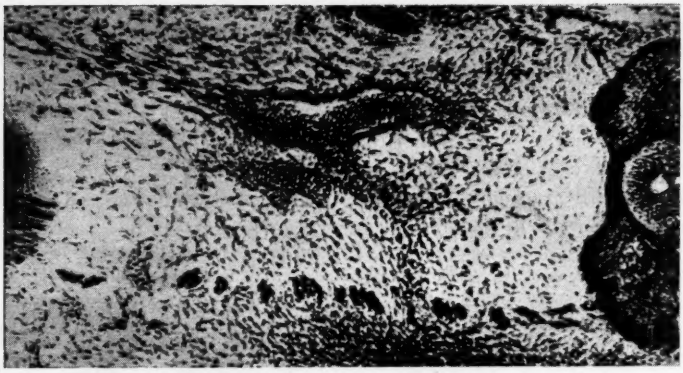
In embryos of 13 days gestation (10–11 mm.) there is a considerable advance in the differentiation of the hypoglossal nucleus which can now be distinguished with some certainty from the adjacent and lateral viscero-motor column. Anteriorly its limit is sharply defined but posteriorly the hypoglossal nucleus is still continuous with the cervical somatic motor column and when the fasciculi are traced from the nervous system the hypoglossal bundles of fibres are arranged in a continuous series with the cervical rootlets, being



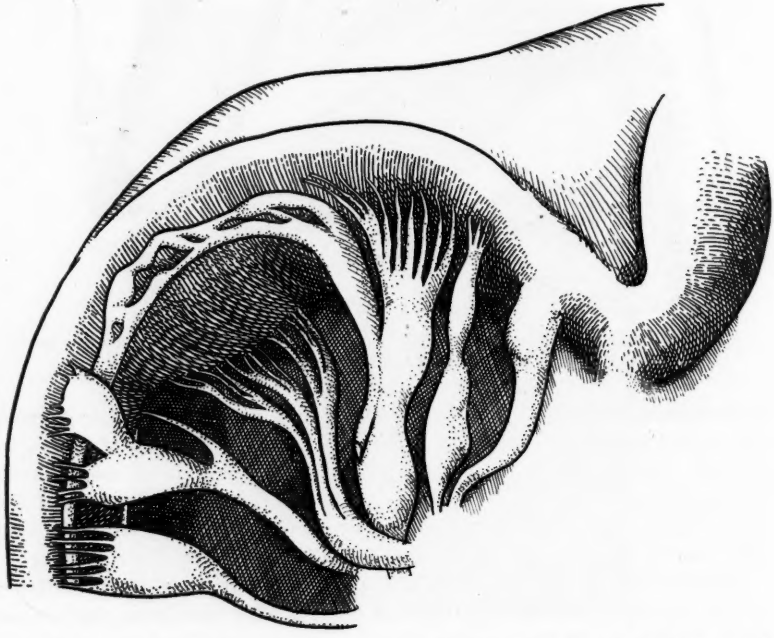
Text-fig. 3. Reconstruction of the origins of the last four cranial nerves and the first two cervical nerves in a 10 mm. rabbit embryo.

distinguishable from them only by the connexions of the latter by dorsal root fibres with their dorsal root ganglion (see Text-fig. 4). The cells of the vago-accessory complex at the level of the origin of the hypoglossal rootlets now show peripheral fibres which are easily to be followed into the trunk of the vagus. But in no section do any of the fibres pass towards the hypoglossal nerve, and there is no appearance of a dorsal hypoglossal root or ganglion.

In the 16-day embryo (17·2 mm.) the hypoglossal fibres are now in intimate contact with the differentiating lingual musculature. Otherwise there is no essential difference in the peripheral course of the nerve from the conditions found in the 13-day embryo (see Text-fig. 5). There is further differentiation



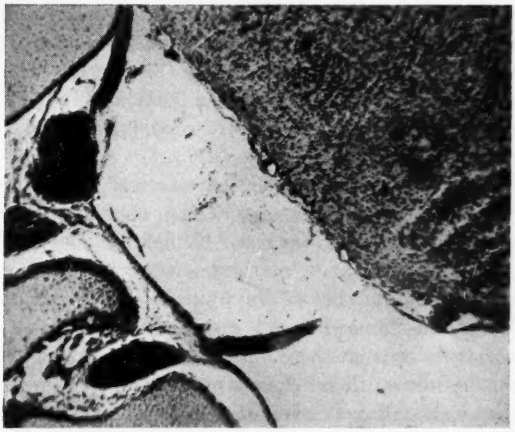
Text-fig. 4. Transverse section of the rootlets of origin of the hypoglossal nerve and the first cervical nerve in a 10 mm. rabbit embryo. Ranson pyridine silver. × 90.



Text-fig. 5. Reconstruction of the origins of the last four cranial nerves, the facial nerve and the first two cervical nerves in a 17·2 mm. rabbit embryo.

of the vago-accessory ganglionic cells, and clumps of ganglionic cells of the cranial part of the accessory are found lying dorso-medially to the spinal accessory nerve bundle. None of these vago-accessory ganglionic cells sends fibres elsewhere than into the vago-accessory nerve complex and there is no appearance of a connexion with hypoglossal fibres which might be regarded as evidence for some of the sensory cells being Froriep ganglion cells intermingled with the vago-accessory ganglia.

Subsequent stages of foetal material examined (22·5, 30, 35, 45 and 56 mm. embryos) showed no material difference in the peripheral relations or connexions of the hypoglossal nerve. Central differentiation of the hypoglossal and adjacent nuclei proceeds rapidly in these later stages but details of this



Text-fig. 6. Frontal section of a hypoglossal root (in lower part of section) in a 56 mm. rabbit foetus. The hypoglossal root is passing through the superior condylar foramen and has no dorsal root, or any connexion with the vagal fibres or the jugular ganglion (in the upper part of the section). Sections 30 μ. $\times$ 30.

differentiation have been well described by Kimmel and need not be considered here. In none of the embryos was a dorsal root or dorsal root ganglion found (see Text-fig. 6) and all the fibres in the hypoglossal roots could be traced back to their origin from the ventro-lateral groove on the medulla. As the embryos get older the vago-accessory ganglia become more circumscribed and finally are represented by the two ganglia (jugulare and nodosum) characteristic of the adult nerve, and at no stage is there any communication between either of these ganglia and the hypoglossal nerve. In his description of the development of the vago-accessory complex Kimmel suggests that some of these cells which can be found along the intracranial portion of the spinal accessory nerve bundle may be Froriep's ganglion. If so, then the term 'Froriep's ganglion' is being used for a group of cells which have no relation

to the hypoglossal nerve, whereas Froriep himself described dorsal hypoglossal ganglia, and it would seem essential to reserve his name as descriptive for the dorsal root ganglia of the hypoglossal nerve which are found in other mammals.

Hypoglossal nerve in newly born rabbit

The hypoglossal nucleus. In the full-term rabbit the nucleus extends through the medulla, appearing first in the posterior part of the metencephalon and reaching, posteriorly, the commencement of the spinal cord. Its anterior boundary can easily be delimited but its termination posteriorly is difficult to define. It is not, however, directly continuous with the cell groups of the grey matter of the ventral horn of the cervical cord. The nucleus is fusiform and consists of large multipolar cells which tend to be grouped into several nuclear subdivisions. The crescentic appearance found on section of the nucleus in some mammals, with a ventral hilum from which the axones of the cells emerge, is not shown by the rabbit. The axones leave the ventral aspect of the nucleus, those arising from the portion of it lying dorsal to the nucleus of Roller passing, for the most part, medial to this nucleus, though a few can be found on its lateral aspect (see Pl. 1, fig. 1). The hypoglossal fibres then pass in parallel bundles through the substance of the medulla and emerge lateral to the pyramid. In their intramedullary course the fibres are closely related to occasional large multipolar cells. No definite statement can be made on the distribution of the axones of these scattered cells, but they do not appear to be joining the bundles of the hypoglossal nerve. In any case the cells are clearly motor in nature and cannot subserve a sensory function. Apart from the possibility that these multipolar reticular cells may contribute to the hypoglossal nerve, there is no contribution to the intramedullary bundles of the hypoglossal axones from any nuclei other than the hypoglossal nucleus itself. Roller (1881), who first described the nucleus associated with his name, thought that the axones from its cells joined the intramedullary course of the hypoglossal nerve. More recently van der Sprenkel (1924) stated that such axones from the nucleus of Roller are distributed to ganglia on the blood vessels of the tongue, being, therefore, preganglionic parasympathetic fibres. Apart, however, from the fact that my sections through the tongue show no ganglionic cells associated with its blood vessels, van der Sprenkel's evidence for the relationship of nucleus of Roller fibres and the hypoglossal nerve is not satisfactory, and my material supports the statements of Vincenzi (1884), Kölliker (1896) and Kimmel (1940), all of whom were unable to find evidence for this relationship. Similarly there is no appearance of cells in the other para-hypoglossal nuclei (nucleus intercalatus, nucleus funiculi teretis, nucleus praepositus) contributing fibres to the hypoglossal fasciculi in their intramedullary course.

The dendrites of the cells of the hypoglossal nucleus form a dense neuropil situated chiefly on the dorsal and dorso-lateral aspects of the nucleus. Some of the dendrites can be traced laterally into the reticular formation, whilst

others pass to the nucleus intercalatus, the dorsal nucleus of the vagus, the fasciculus longitudinalis medialis and, a few, across the raphe to the nucleus of the other side. A large number of the fibres in the neuropil are terminals or collaterals of axones arising from cells elsewhere in the nervous system, but no analysis of them is possible in the available material. I have not been able to locate the so-called 'accessory' nucleus of the hypoglossal nerve, the cells of which, according to Kölliker (1896), do not send their axones out with the hypoglossal nerve. All of the large multipolar cells which constitute the hypoglossal nucleus in the rabbit appear to send their axones into the intramedullary bundles of the nerve, and there is nothing in the newly born rabbit medulla, nor in the adult, to suggest that the hypoglossal nerve has any intramedullary connexions comparable to the mesencephalic nucleus of the trigeminal nerve.

Extra-cerebral course of the nerve. The origin of a radicle of the nerve from the brain-stem is shown in Pl. 1, fig. 2. No trace of a Froriep's ganglion was found in any of the sections. In marked contrast with Tarkhan's description, no ganglion cells were found in the course of the nerve and no collection of sensory cells was found contributing fibres to it (see Pl. 1, figs. 3, 4; Pl. 2, figs. 5, 6). This statement is made after careful examination of complete serial sections through the whole of the hypoglossal nerve in four full-time rabbit foetuses. The material is excellently impregnated and isolated ganglion cells can be found in the peripheral portions of the first cervical nerve, the lingual nerve and the glossopharyngeal nerve. The large ganglia of the vagus, glossopharyngeal and cervical nerves are also well impregnated. In view of the histological condition of this material it is felt that hypoglossal ganglia or isolated ganglionic cells in the course of the nerve could not be missed if they were present. Tarkhan's description is based on a single specimen, isolated by dissection from its surroundings, and it would seem that either the ganglion he describes is abnormal or he has confused some other group of nerve cells, snared off during dissection from an adjacent ganglion, for a peripheral ganglion of the hypoglossal nerve.

In several of the sections fibres from the superior cervical ganglion can be traced to the hypoglossal trunk. Such a group of sympathetic fibres is seen in Pl. 2, fig. 5, and they probably constitute the pathway for some of the vasomotor fibres to the lingual blood vessels. Weddell and his co-workers showed that section of the hypoglossal nerve close to the tongue leads to degeneration of all the nerve fibres ending in the muscles and on the blood vessels but that section of the hypoglossal at the base of the skull causes degeneration of the motor fibres to the muscle though it does not affect the nerves supplying the blood vessels. The inconstancy of the fibres from the superior cervical ganglion to the hypoglossal nerve in the rabbit, as shown both in dissections and in the serial sections, suggests that a number of the sympathetic fibres reach the lingual blood vessels along the alternative route of the lingual artery.

Sensory endings in lingual musculature of the rabbit

Franqué (1890), Pilliet (1890), Förster (1894), Langworthy (1924) and Tarkhan (1936) have stated that muscle spindles are present in the tongue musculature. Sherrington (1894), Batten (1897), Baum (1900), Boeke (1927), Hewer (1935), Carleton (1938), and Weddell *et al.* (1940) were all unable to find them, and to this list I must add myself, for in none of my material was any evidence found for the existence of muscle spindles in the intrinsic or extrinsic musculature of the tongue of the rabbit. This material includes sections in varying planes and thicknesses through the tongue of foetal, newly born and adult rabbits. A few spindles can be found in the infrahyoid muscles, more in the pre-vertebral muscles and a great many in the muscles of the back of the neck (see Pl. 2, fig. 8). There are several histological criteria by which muscle spindles may be recognized: (1) the nature of the intrafusal muscle fibre or fibres; (2) the presence of a capsule and of a periaxial lymph space; (3) the peculiar relationship existing between the muscle fibres and the nerve fibres; and (4) the epilemmal nature of the sensory nerve ending. The recognition of a spindle when all these criteria are fulfilled is a simple matter, and on transverse or longitudinal section through the spindle there can be no doubt. Such spindles are found in the muscles of the back of the neck of the rabbit (see Pl. 2, fig. 8). When all of the criteria are not fulfilled, and, as Hines (1930) has shown, they frequently are not, the decision whether or not muscle spindles are present is difficult, inevitably involving some subjective opinion. But in the rabbit's tongue (see Pl. 2, fig. 7) there can be no confusion for there are no structures in silver sections which give any appearance remotely resembling muscle spindles in any of their characters. In spread preparations, as suggested by Weddell and his co-workers, perivascular nerves might give rise to some confusion. But in sections there can be none. Tarkhan evidently realized that the structures described by him as muscle spindles are not typical for he wrote: 'die charakteristischen Strukturen einer Muskelspindel...in Schnitten nicht immer so deutlich zum Ausdruck kommen wie in Zupfpräparaten.' As a general statement this is only true when the sections are not through the main transverse or longitudinal axis of the spindle and it cannot be used to explain the complete absence of structures with any of the characters he mentions: 'Kapsel, perilymphatischen Raum, spiralig aufgerollte Nervenfasern'—in any of my sections, many of which are serial, through the tongue muscles of the rabbit, while other muscles in the same sections possess unmistakable muscle spindles with all of the attributes of true muscle spindles plainly apparent.

In the absence, then, of muscle spindles in the tongue musculature we must look elsewhere for the histological basis of its proprioceptive mechanism, if it possesses one. Boeke, aware of the absence of muscle spindles, suggested that the tongue is supplied with deep sensibility by means of fibres which terminate as end-nets in the connective tissue interstices of the tongue musculature, and

which he derived from the hypoglossal nerve. He supposed the mechanism to be similar to that described by Dogiel and by Huber in the eye muscles. Woollard (1931), in his paper on the innervation of the extrinsic muscles of the eye, made a similar suggestion. He wrote: 'There is some reason to suppose that a similar kind of innervation [to that in the ocular muscles] obtains in the tongue, perhaps also in the muscles of the face and the muscles of mastication.' In my material ramifications of nerve fibres in the lingual connective tissue can be found, but in serial sections it is impossible to be certain that such fibres are not collaterals of larger fibres passing to muscle. Certainly no specialized endings of a sensory nature can be found on them. A general argument against the use of silver impregnation is the frequency with which reticular connective tissue fibres are stained and it may be that Boeke's endings and the fibres seen in my material are, in part, artefacts. They can, however, frequently be traced to other nerve fibres, and the de Castro technique, in my experience, is much less likely to give rise to confusion in this regard than the Bielschowsky method used by Boeke. More definitely against Boeke's suggestion, however, is the work of Weddell *et al.* who used methylene blue in vivo. Their material showed no sensory endings of any sort in the intrinsic lingual musculature, and in view of the beauty and clarity of their material I think this result must be accepted. In the genioglossus muscle, however, at its attachment to the mandible these workers did find endings which consisted of a thick coil of axis cylinder surrounded by enlarged neurilemmal cells. In view of their work I have carefully examined my material and have found several atypical endings in both the genioglossus and the hyoglossus muscles in a position corresponding to that described by them (see Pl. 2, fig. 9). These endings are not easy to interpret, at any rate in rabbit material, and they are so rare (only two or three being found in each muscle) that it is difficult to accept them as being adequate in themselves alone for the mediation of lingual proprioception if the tongue possesses a deep sensibility equivalent to that of most other somatic muscles. Whatever may be the explanation of these endings, however, the work of Carleton, and of Weddell and his collaborators, when taken into consideration with my results, seems to make it quite definite that those investigators who have described muscle spindles in rodent tongue are mistaken.

DISCUSSION

Histological and embryological observations cannot in themselves solve the problem of the proprioceptive innervation of the tongue and, in any case, those reported above, being limited to the rabbit, are only of real significance when this mammal is considered. The rabbit, however, has been largely used in the investigation of the problem, and here my results show quite definitely that any proprioceptive fibres from the tongue cannot have their cell bodies in the trunk of the hypoglossal nerve or in a hypoglossal dorsal root ganglion. Further, the hypoglossal nerve fibres inside the brain stem appear to have no

direct connexions other than with the hypoglossal motor nucleus and, possibly, isolated multipolar motor cells in the dorsal reticular region. Tarkhan's description of a peripheral hypoglossal nucleus is, therefore, incorrect, and any proprioceptive fibres from muscles supplied by branches of the hypoglossal nerve must reach the nervous system, if they exist, by pathways other than the hypoglossal rootlets themselves.

Barron (1936) was unable to find any action potentials in the hypoglossal nerve of cats, rabbits and rats following stretching, deforming, touching, or burning of the tongue, or by the application to it of acids, alkalis or salts. No impulses arising in the periphery were ever recorded in the hypoglossal nerve in his experiments. On the other hand, he was able to record impulse discharges from the lingual nerve when the tongue was stretched, but these discharges, when recorded in single fibre preparations, did not have as high an initial frequency as that described by Matthews (1933) for muscle spindles and, further, the end organs producing them adapted more rapidly. Barron concluded that sensory fibres from the intrinsic tongue musculature do not exist in the hypoglossal nerve but pass centrally by the lingual nerve and have their cell bodies in the trigeminal ganglion or in association with the mesencephalic nucleus of the trigeminal nerve. The work of Carleton, and of Weddell and his collaborators, however, demonstrated complete degeneration of endings in relation to the tongue musculature on section of the hypoglossal nerve. These results, which agree with the earlier observations by Boeke, seem quite definitely to rule out the possibility that the impulses recorded by Barron from the lingual nerve can have their origin in lingual muscular proprioceptors. That they may be arising from endings of the lingual nerve in the submucosa of the tongue cannot be excluded, although my material does not show any reason for assuming the presence of specialized endings in this position. It appears, therefore, that a trigeminal afferent pathway from the musculature of the tongue can be excluded. Nevertheless, Barron's inability to record afferent impulses from the hypoglossal nerve is of considerable importance, and his results, if verified, suggest that either the hypoglossal nerve possesses no afferent fibres, or very few, or that his electrodes were on a portion of the hypoglossal nerve beyond the point at which proprioceptive fibres leave it.

There remain, therefore, the possibilities that fibres pass centrally by way of a communication between the hypoglossal nerve and other cranial nerves or anterior cervical nerves, or that the sympathetic fibres to the tongue may be concerned in the central transmission of impulses from the tongue musculature. The latter suggestion cannot be automatically ruled out, but it is contrary to what is known of the distribution of proprioceptive fibres to other voluntary musculature and is not supported by any experimental evidence beyond the fact that the cervical sympathetic chain appears to be involved in the pupillary dilatation observed by Downman (1939) on faradic stimulation of the hypoglossal nerve and analysed by Weddell and his co-workers as

representing most probably a local autonomic reflex. My material shows no connexion between the hypoglossal nerve and the glossopharyngeal and vagus nerves of the rabbit, making the existence of a centripetal pathway from the tongue by way of one of these cranial nerves very unlikely.

We are left, then, with the possibility that any afferent fibres in the hypoglossal nerve which might mediate the proprioception of the tongue must pass to the nervous system by way of the upper cervical dorsal roots. Yee *et al.* (1939) have shown that removal of the first cervical dorsal root ganglion in the rabbit results in the degeneration of a small number (1–5 %) of medium sized myelinated fibres in the hypoglossal nerve. These fibres pass to the hypoglossal nerve through the communicating branch from the first and second cervical anterior rami which join the descendens hypoglossi and pass up to the hypoglossal nerve itself. Since stimulation of the anterior ramus of the first cervical nerve never causes contraction of the tongue musculature, these workers concluded that these fibres of communication must be afferent. When this work is considered in the light of the contribution of Weddell and his collaborators, it seems that we must accept a very small afferent component in the peripheral hypoglossal nerve, concerned only with the extrinsic muscles which possess a few atypical endings. The cell-bodies of this small afferent component are situated, in the rabbit, in the first cervical dorsal root ganglion. In the rhesus monkey Corbin *et al.* (1937) found evidence for a small contribution (*circa* 1 %) to the hypoglossal nerve from the second cervical dorsal root ganglion. This caudal shift in a primate of those few sensory cells giving origin to afferent fibres connected with the hypoglossal nerve can be associated with that phylogenetic trend which is resulting in the disappearance of the first cervical dorsal root ganglion in the higher members of this mammalian order. On the other hand, Corbin & Harrison (1938) have been puzzled by their inability to find evidence for a contribution to the hypoglossal nerve from dorsal root ganglia of the cervical nerves in the cat. The disparity, however, between the results for the rabbit and the monkey on the one hand and the cat on the other can be explained by the fact that dorsal hypoglossal roots are to be found in the cat. I have not yet checked the matter histologically, but if these dorsal roots possess functional ganglion cells the cat, like the pig and the cow, has retained the sensory component of the hypoglossal nerve and the cells of origin of this component constitute true Froriep's ganglia. In other mammals Froriep's ganglia, in a functional sense, are still present, but morphologically they have disappeared as separate entities, having become incorporated in the first or, when this is absent, the second cervical dorsal root ganglion. How this incorporation is brought about is not clear, but the developmental history of the hind-brain region in the rabbit does not show a separate Froriep's ganglion at any stage. Its cells, then, must be included in the developing first cervical dorsal root ganglion from the stage of its earliest differentiation from the occipito-spinal portion of the neural crest.

Finally, it must be stressed that the afferent component in the hypoglossal nerve is very small. It does not seem possible that the few atypical endings in the attachment to bone of the extrinsic muscles of the tongue are sufficient to give the reflex control over the lingual musculature required in sucking, mastication and trituration and, in some mammals, prehension of food. Possibly the superficial sensation of the tongue has come to have a particular role in these functions, eliminating, or acting vicariously for, specialized muscular proprioceptors. In man, with the added complexity of the tongue movements in speech, the absence of a rich afferent lingual innervation is even more surprising. My histological material of the human tongue, however, although it is sparse and not in extensive serial sections, agrees with the results reported by Hewer (1935) who was unable to find muscle spindles in the tongue of the human foetus.

SUMMARY

1. At no stage in the development of the rabbit hypoglossal nerve could a dorsal root or a ganglion of the Froriep's type be found.

2. Muscle spindles, or other specialized sensory endings, could not be found in the intrinsic muscles of the rabbit's tongue. In the extrinsic muscles, although muscle spindles are absent, a few atypical endings can be found close to the attachment of the muscles to the mandible and hyoid bone.

3. The macroscopic and microscopic anatomy of the rabbit hypoglossal nerve, when considered in relation to the relevant experimental findings, appears to demonstrate that any afferent fibres in the nerve can reach the central nervous system only by way of the upper cervical dorsal roots.

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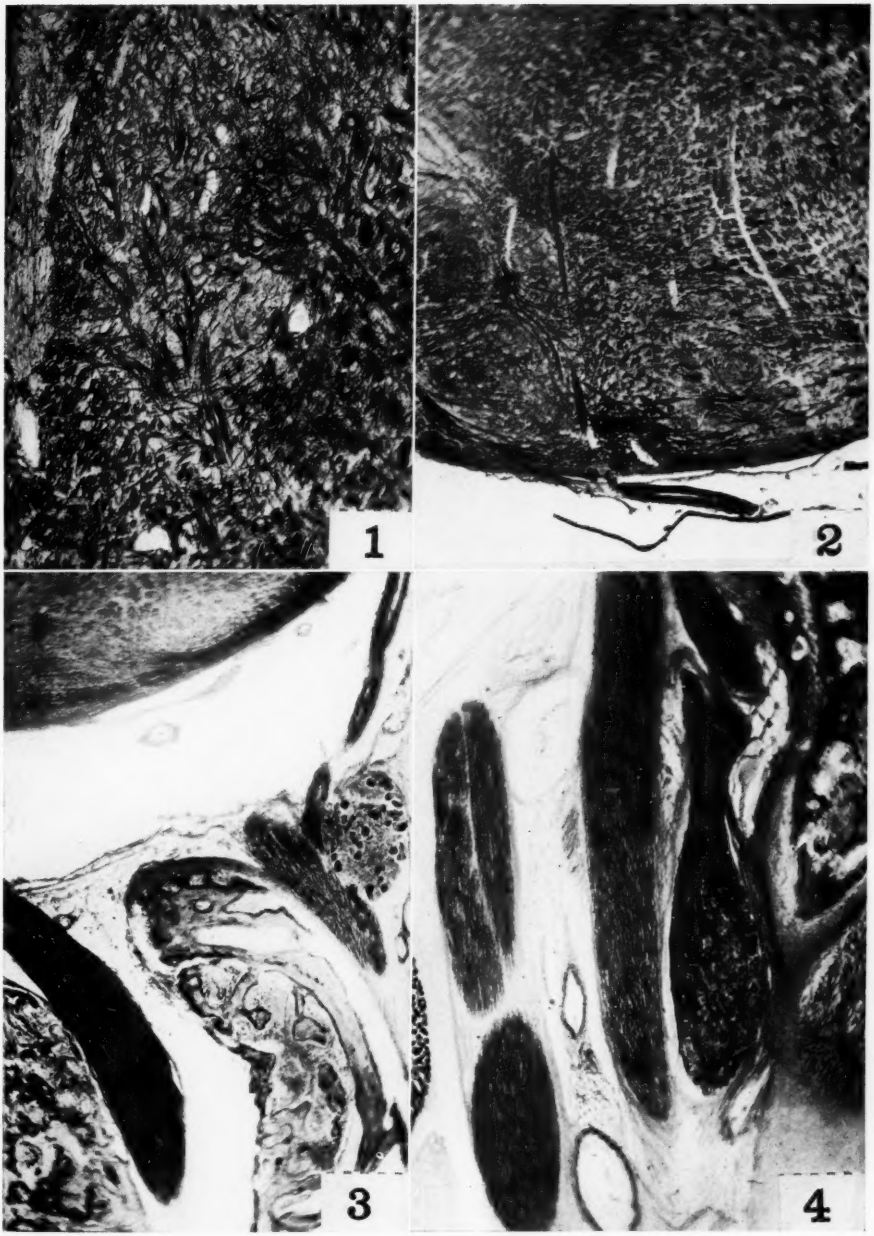

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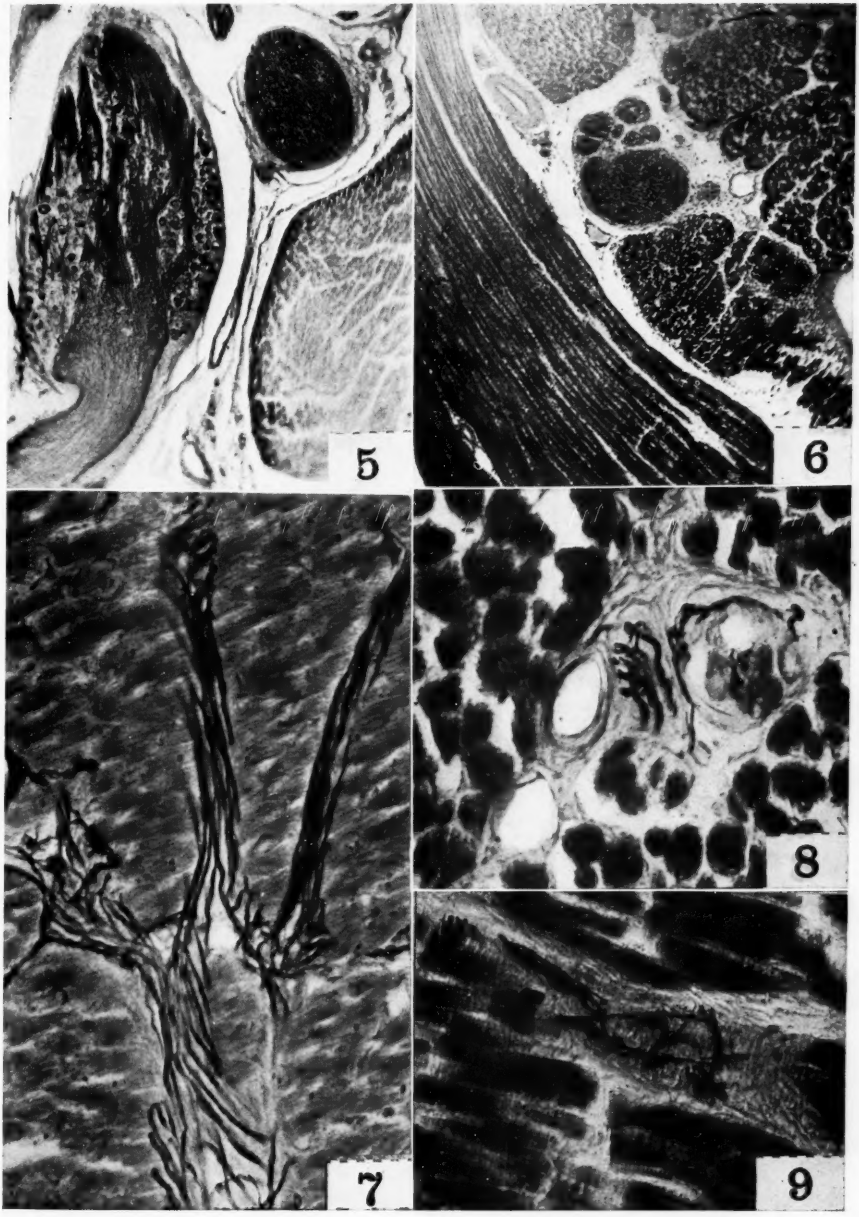

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EXPLANATION OF PLATES 1 AND 2

PLATE 1

Fig. 1. Hypoglossal nucleus and nucleus of Roller (pale) in a newly born rabbit. de Castro technique. × 80.

Fig. 2. Origin of hypoglossal fasciculus in a newly born rabbit. de Castro technique. ×50.

Fig. 3. Root of hypoglossal nerve passing through the foramen condyloideum superius. No nerve cells can be seen in the hypoglossal fibres (left lower part of section) though they are clearly shown in the ganglion jugulare of the vagus (upper right part of section). de Castro technique. ×50.

Fig. 4. Two hypoglossal roots lateral to trunk of vagus and ganglion petrosum of the glossopharyngeal nerve. Note absence of nerve cells in the hypoglossal roots and absence of communications between it and the vagus. de Castro technique. ×50.

PLATE 2

Fig. 5. Hypoglossal nerve trunk related to lateral aspect of ganglion nodosum of the vagus nerve. There are no nerve cells in the hypoglossal nerve though many can be seen in the ganglion nodosum. The small bundle of fibres joining the lower aspect of the hypoglossal nerve can be traced to the superior cervical ganglion. There is no appearance of a communication with the ganglion nodosum. de Castro technique. ×50.

Fig. 6. Hypoglossal nerve in the substance of the tongue. No nerve cells are present in it. de Castro technique. ×50.

Fig. 7. Ramifications of hypoglossal nerve fibres in intrinsic tongue musculature. No muscle spindles are present. de Castro technique. ×350.

Fig. 8. Transverse section of muscle spindle in sub-occipital muscles. From same section as previous figure. de Castro technique. ×450.

Fig. 9. Atypical ending in the origin of the hyoglossus muscle. ×450.

THE PATTERN OF CUTANEOUS INNERVATION IN RELATION TO CUTANEOUS SENSIBILITY

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THE purpose of this paper is to present histological findings which indicate the fundamental pattern of cutaneous innervation in vertebrates, as illustrated by an elasmobranch fish, the rabbit, the rhesus monkey, and Man. These findings are compared with clinical observations on Man and discussed in the light of Head's theory of protopathic and epicritic sensibility.

MATERIAL AND METHODS

Histological. Whole preparations of considerable areas of skin from the flank of an elasmobranch fish, *Acanthias vulgaris*, were impregnated with silver by a modified Bielschowsky-Gros method. This was carried out by Dr P. Glees of the Department of Human Anatomy, Oxford, to whom I am much indebted for the loan of preparations.

Whole preparations of skin from the ears of albino rabbits were stained with methylene blue by the technique of local injection (Weddell *et al.* 1940). Minor modifications have been introduced, following the observation that the most complete and uniform staining over a wide area resulted from the vasodilatation following cervical sympathetic neurectomy. To produce the same effect, a local anaesthetic (1 % procaine hydrochloride) was dissolved in a concentration of 0·015 % methylene blue in normal saline solution. This is found to give consistent staining over wide areas in about half an hour, and it is possible by this method to stain nerves uniformly throughout the skin of the dorsal or ventral surface of the ear.

Nerves in the skin of the forearm and finger-pad of the monkey were stained in a similar manner.

In Man unexpected difficulties were encountered in obtaining consistent and uniform staining. This was found to be due in part to the rapid concentration and excretion of the dye by the sweat glands, and in part to the thickness of the skin. The addition of atropine sulphate to the injected solution delays the excretion, and to some extent the concentration, of the dye by the sweat glands. In most cases good results can be obtained when the concentration of the dye is increased to 0·03 % in normal saline solution containing procaine hydrochloride 1 % and atropine sulphate 1/10th grain per 100 c.c.

Clinical. The areas of sensory loss of touch, pain, cold and warmth were mapped out in patients suffering from peripheral nerve injuries which included root lesions, plexus lesions, and nerve trunk lesions. The test objects used were

von Frey hairs for touch, sharp needles for pain, and plane copper discs, 1 cm. in diameter, which could be maintained at constant temperatures, for warmth and cold.

Experimental. The dorsal nerve at the base of the ear in albino rabbits was resected in ten cases and partially sectioned in four cases. The partial section was performed under direct vision through a dissecting microscope by a single cut with iridectomy scissors. Approximately one-quarter of the trunk was divided. Care was taken to avoid damaging vessels running in the connective tissue sheath surrounding the nerve. Various intervals, ranging from 12 hr. to 4 days, were allowed for nerve degeneration in the case of total resection; 2 days were allowed to elapse following partial nerve section.

In Man the radial nerve was blocked in the lower third of the forearm in a normal subject who is experienced in the perception of sensory stimuli, by the infiltration of 2 % novocain containing 1 in 20,000 adrenalin. The areas of loss of pain and touch sensibility were mapped out. The same nerve was similarly blocked on a subsequent occasion in the lower third of the arm and the areas of loss of pain and touch sensibility again mapped out. The test objects used were sharp needles and von Frey hairs. The nerve blocks and sensory tests were carried out by Dr L. Guttmann, to whom I am much indebted.

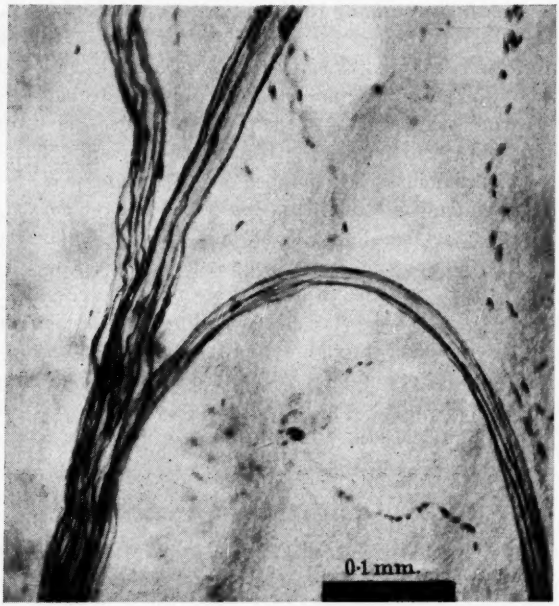
Attempts were made to see stained nerve fibres and endings in the intact human skin by direct vision through a microscope. A 16 mm. objective fitted with an oil-immersion dipping cone and oblique illumination were used. The source of illumination was a low-voltage filament lamp, light from which was concentrated into a circle 1 mm. in diameter by a carefully corrected optical system.

HISTOLOGICAL OBSERVATIONS

In *Acanthias vulgaris* there is a cutaneous nerve plexus disposed in two main layers through the thickness of the skin. Single fibres travelling in the deeper nerve trunks, when traced peripherally, are seen to change direction and enter the cutaneous nerve plexus in which they dichotomize repeatedly as they approach the skin surface. The terminal ramifications of a single fibre become evenly distributed over a wide, approximately circular, area. The final branches of the fibres which were followed were found to end freely beneath and between the cells of the basal layer of the epidermis. Neighbouring fibres undergo a similar dichotomization through the cutaneous nerve plexus, and the terminal ramifications of any one fibre are always evenly interlocked with those of surrounding fibres. The method of distribution of the terminal ramifications may be compared with the branching and interlacings of a cultivated vine.

In the ear of the rabbit the cutaneous nerve trunks are arranged in a pattern which can be seen macroscopically after staining with methylene blue; they follow the venous pattern closely. A large nerve trunk enters the root of the ear, lying on the lateral side of the main dorsal vein. This soon gives rise

to large branches which pass up the margins of the ear. The central nerve trunk continues towards the apex of the ear, giving off lateral branches at intervals. These pass towards the margins of the ear and divide into ascending and descending trunks which inosculate with other trunks derived in a similar manner. The pattern is complicated at various points by the formation of intermediate arcades and inosculations which occur between the marginal and central trunks (Text-fig. 1). In the marginal nerve trunks and intermediate

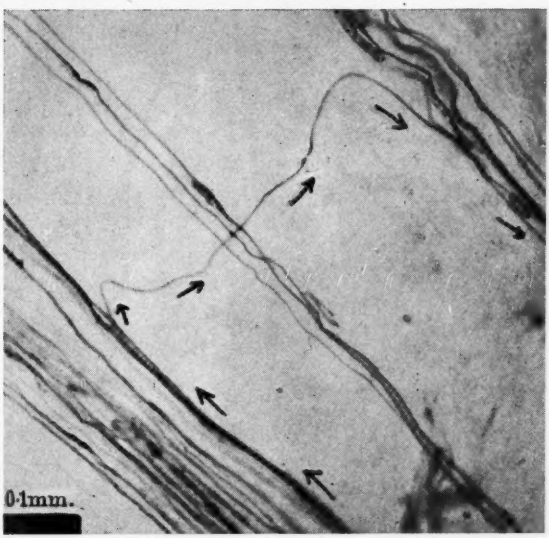


Text-fig. 1. Photomicrograph showing change in direction of nerve fibres derived from a main nerve trunk in skin from the dorsum of the ear of the rabbit. Methylene blue preparation.

arcades the nerve fibres pass in both directions, some fibres passing distally and others returning proximally towards their terminations. No nerve fibres within the nerve trunks were actually seen to dichotomize, but it is possible that this may occur occasionally. The central nerve trunk is composed of a number of fasciculi. Fibres occasionally leave one fasciculus to join another, in which they may continue distally or return proximally before leaving the main nerve trunk (Text-fig. 2). In other words, not every fibre in the main nerve trunk is necessarily passing in a distal direction; on the contrary, some may be running a recurrent course. In this connexion it has been noticed on several occasions that stimulation of the distal, as well as of the proximal ends, of the blue severed nerve trunks seen in the subcutaneous tissue after the

removal of skin stained with methylene blue gave rise to nociceptive reactions in lightly anaesthetized animals. These reactions take place frequently when the distal end of the central nerve trunk is stimulated beyond the point where it has given rise to the marginal nerve trunks, but only occasionally if stimulated before it has given rise to these trunks.

It will be observed from this general account that there is no question that the nerve fibres of a main trunk or of its branches approach from one direction, and in approximately parallel formation, the area of skin which they are destined to supply, and then deploy in an even pencil of fibres over the area.



Text-fig. 2. Photomicrograph showing a nerve fibre passing from one fasciculus to another in the main nerve trunk of the dorsal nerve in the ear of the rabbit and running a recurrent course. Methylene blue preparation.

On the contrary, to use a botanical simile, the pattern of innervation resembles the reticulate venation seen in certain types of leaves in contrast to the parallel venation seen in others.

Nerve bundles from the main nerve trunks give rise to branches which pass up from the subcutaneous layers towards the skin surface and distribute fibres to the cutaneous nerve plexus (Pl. 1, fig. 1). The latter is disposed in two main layers through the thickness of the skin. On entering the plexus, the fibres undergo repeated dichotomization. The cutaneous nerve plexus is formed by the inosculation of the two layers of dichotomizing nerve fibres arranged in meshwork patterns which enclose polygonal areas of approximately hexagonal shape (Text-fig. 3). Hair follicles lie for the most part at

the centre of the superficial polygonal areas enclosed by the nerve-fibre meshwork. The terminal nerve-fibre ramifications which supply the hairs approach them in several directions from the periphery.

In the case of the rabbit, there are two modes of nerve-fibre termination. Some fibres end around the hair follicles and innervate the hairs; others end in nerve nets immediately beneath the epidermis. From these nets, fine beaded endings arise which are disposed below and between the cells of the deeper layers of the epithelium. The branches of an individual fibre traced throughout

Text-fig. 3. Photomicrograph showing the neural topography in the skin from dorsum of the ear of the rabbit. Methylene blue preparation. ×47.

its ramifications in the cutaneous plexus bear endings of only one variety. Beneath the epidermis, fibres can be seen arising from nerve bundles, and in some cases from the deeper layer of the cutaneous nerve plexus, forming nerve nets around the larger blood vessels. Fine fibres leave the nets and ramify in the walls of the vessels, and the capillary blood vessels in the subcutaneous areolar tissue at the base of the dorsum of the ear are accompanied by fine beaded nerve fibres derived from similar nerve nets.

After total resection of the dorsal ear nerve, the fibres supplying the hair follicles and giving rise to the subepidermal nerve nets are found to be undergoing degenerative changes. All such fibres in the skin from the centre of the

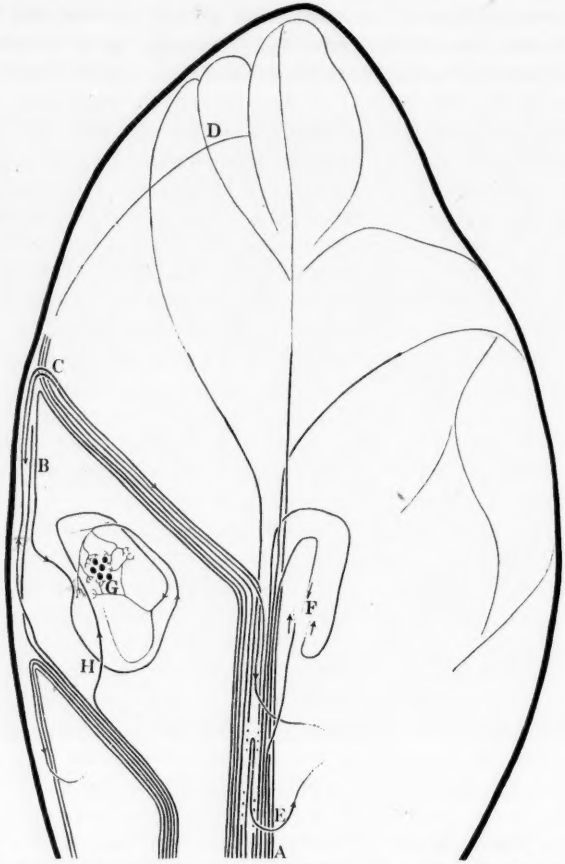
dorsum of the ear were found to exhibit histological evidence of degeneration at the end of 2 days; these changes were clearly evident in the nerve trunks and at the fibre terminations.

After partial section of the dorsal ear nerve the degenerating fibres stand out in contrast to the normal fibres. In one instance it was possible to trace a single degenerating fibre to its termination around hair follicles. This fibre was found to run through the main and subsidiary nerve bundles without branching, although it changed its direction several times. Dichotomization began as soon as the fibre entered the deep layer of the cutaneous plexus. The branches continued to multiply through the plexus and were distributed over a wide, approximately circular, area, the greatest diameter of which was 1 cm. The number of hair-follicle groups supplied by branches from this single fibre was in the neighbourhood of three hundred, and a group of hair follicles may contain up to ten hairs. The terminal ramifications of every main fibre so traced was found to remain independent of those from other fibres. It is important to note that each hair follicle group is supplied by branches from at least two main nerve fibres (Pl. 1, fig. 2). This also applies to the individual hairs. The nerve fibres leaving the superficial plexus usually break up into a number of branches on their way to the hairs, so that a single hair may be innervated by as many as fifteen terminal ramifications. After partial dorsal nerve section, degenerating nerve fibres can be seen over the whole extent of the dorsum of the ear of the rabbit. The plan of innervation of the skin of the ear of the rabbit is summarized in Text-fig. 4.

In skin taken from the pad of the thumb of a rhesus monkey there was found to be a cutaneous nerve plexus disposed in two main layers. Its pattern is, in general, similar to that seen in skin from the ear of the rabbit. Arising from the superficial layer are fibres of which each bears one or more endings. The endings borne by a single fibre arising from the cutaneous nerve plexus are always of the same type. The following types of ending have been identified: Meissner's corpuscles, Merkel's discs, Krause's end-bulbs, and fibres giving rise to nerve nets situated immediately beneath the epidermis. Fine terminal fibres arise from the net and end beneath and between the cells of the deep layer of the epidermis, and the endings derived from the nerve nets can be seen to lie in the same optical plane (4 mm. dry objective) as the cells of the deep layers of the epidermis (Text-fig. 6) The nerve nets from a single fibre are distributed over an approximately circular area. The nerve fibres from which they arise remain single and unbranched as far as they can be traced through the cutaneous nerve plexus. The nerve net derived from any one fibre is interlocked with those arising from neighbouring single unbranched fibres (Text-fig. 5). There is in no instance any visible fusion between the interlocked ramifications of nerve nets derived from separate fibres; on the other hand, there is continuity between the branches of the nerve net derived from a single nerve fibre. The area of skin covered by a net arising from a single nerve fibre in the thumb of the rhesus monkey is approximately 1·5 mm. in diameter.

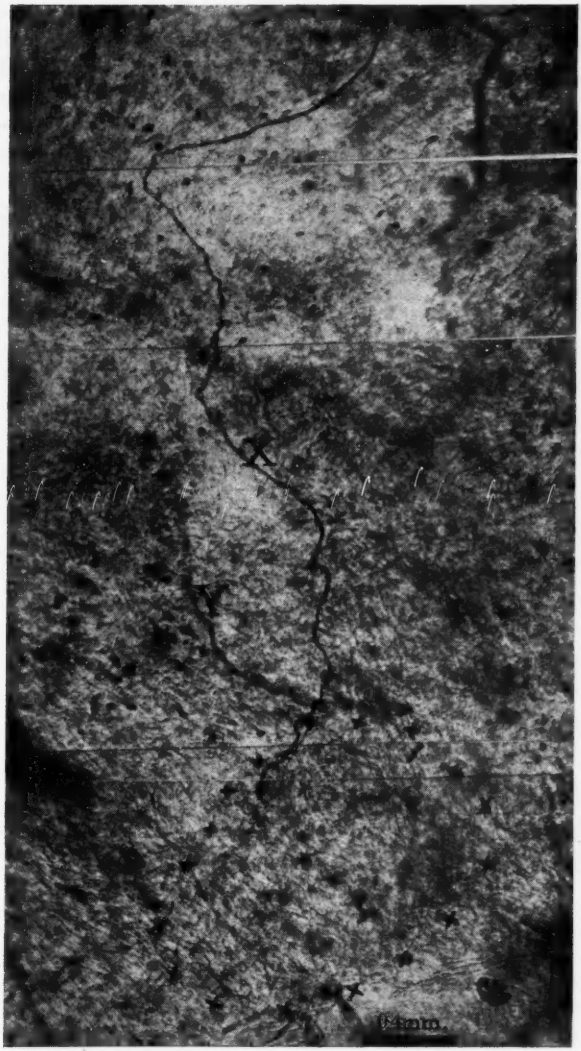
HISTOLOGICAL OBSERVATIONS IN MAN

Direct observation. Direct observation of nerve fibres and endings in the intact, vitally stained skin of the human arm and hand proved unsatisfactory for two reasons. It was found extremely difficult to keep the arm and hand



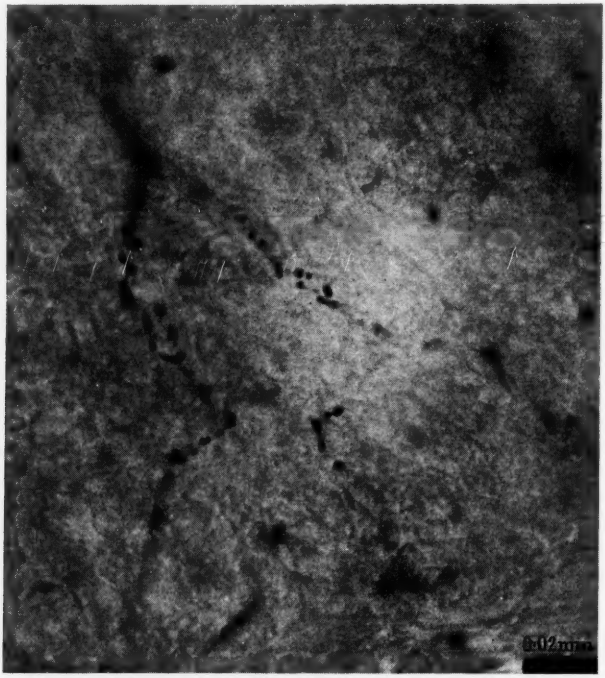
Text-fig. 4. A line drawing summarizing the general pattern of the distribution of the nerve fibres in skin from the dorsum of the ear of the rabbit. A, main nerve trunk; B, marginal trunk; fibres are passing in both directions; C, nerve fibres inosculating; D, intermediate arcade; E, nerve fibre which has run a recurrent course in the main nerve trunk; F, showing a mode of innervation of a unit area of skin; G, hair follicle group; H shows innervation of a hair follicle group by two fibres arising from subsidiary nerve bundles and passing through the cutaneous nerve plexus.

sufficiently still under the microscope for detailed observations to be made, and it was not found possible to see the details of any structure situated more deeply below the surface of the skin than the capillary loops in the dermal



Text-fig. 5. Photomicrograph showing a fine nerve fibre *X* emerging from the cutaneous nerve plexus from the pad of the thumb of a monkey and giving rise to a superficial nerve net. The area over which the nerve net is distributed is outlined by the ink crosses. It is 1·5 mm. in diameter. An interlocking nerve net is derived from the nerve fibre marked *Y*. Methylene blue preparation.

papillae. Half an hour after the injection of the dye into the skin of the forearm of a normal human subject (G. W.) fine blue nerve fibres arranged like a spireme were seen around some of the capillary loops in the dermal papillae, but it was not possible to trace the course of the fibres owing to the greater thickness of the interpapillary skin. Fine blue dots could also be seen over the whole stained area lying just beneath the surface layers of the epidermis, but, again, their connexions could not be traced. The dots were, however,



Text-fig. 6. Fine beaded endings lying between the cells of the deep layer of the epidermis derived from a superficial nerve net in the pad of the thumb of a monkey. Methylene blue preparation.

similar in all respects to the beaded terminals derived from superficial nerve nets seen immediately beneath the epidermis in cleared specimens of skin. The ducts of the sweat glands could be seen as blue coiled tubes passing through the surface layers of the epithelium. They were dark in colour, particularly if the injected dye solution contained neither local anaesthetic nor atropine, and they could then be seen intermittently discharging drops of blue fluid on to the skin surface. After discharging the fluid the ducts became pale.

Biopsy material. The skin to be described from the first subject was taken

some years ago during work done in collaboration with the late Prof. H. H. Woollard. A cold spot had previously been mapped out on the skin of the forearm (H. H. W.) by the method described by Woollard (1935). A full thickness of skin 0·5 cm. square which included the cold spot was removed and stained supravitally with methylene blue. Histological examination showed a cutaneous nerve plexus resembling that seen in the monkey. Close beneath the surface ink mark which indicated the position of the cold spot, two groups of endings resembling Krause's end-bulbs were seen to be borne upon terminal ramifications of thick nerve fibres arising from the superficial layer of the cutaneous plexus. The two groups of endings were 0·3 cm. apart. It was not possible to ascertain whether they were borne on branches of the same fibre or on independent fibres; they were, however, derived from fibres which could be traced for some distance through the superficial layers of the cutaneous nerve plexus and which themselves gave rise to further branches. Owing to the limited size of the preparation the latter could not be traced to their peripheral destinations. The endings resembling Krause's end-bulbs were situated approximately 1 mm. below the surface of the skin. Meissner's corpuscles, hair sheath endings, nerve fibres passing to blood vessels, and nerve fibres which gave rise to more superficially placed nerve nets, were seen to arise from nerve fibres which ran an independent course within the cutaneous plexus. The map of the neural topography underlying the cold spot (Pl. 2) illustrates these findings. This map was made by placing together a number of photomicrographs which followed the course of the nerve fibres to their endings through varying optical planes.

Skin from a second subject was stained and removed from the forearm of a patient (J. A.) attending the skin department of the Radcliffe Infirmary, Oxford, for psoriasis elsewhere on the body. This piece of skin measured 1 by 0·5 cm. A similar cutaneous nerve plexus was seen in it. From the plexus fibres arose which ended around hair follicles, other fibres giving rise to Meissner's corpuscles, and still other fibres to superficially placed nerve nets situated beneath the epidermis. No other types of organized ending were seen. The endings derived from the nerve nets were particularly well stained and could be seen as a carpet of beaded terminals lying between and beneath the cells of the deep layers of the epidermis.

In order to determine the neurohistology of skin from which only one modality of sensation could be aroused, the pattern of sensory loss was outlined in a patient (Pte At.) with a sciatic nerve lesion. The area from which pain could not be aroused was smaller than that from which touch could not be aroused, and this in turn was smaller than the areas over which temperature sensibility had been lost. A piece of skin 2 by 3 cm. was stained and removed from a zone in part of which pain only could be aroused and in part of which both touch and pain could be evoked. In the area from which pain alone could be aroused fine nerve fibres were seen giving rise to superficial nerve nets; no thick nerve fibres or organized endings were seen. On the other hand, in the

area of skin in which both pain and touch could be aroused, the cutaneous nerve plexus was seen together with thick nerve fibres ending around hairs and thinner fibres giving rise to superficial nerve nets.

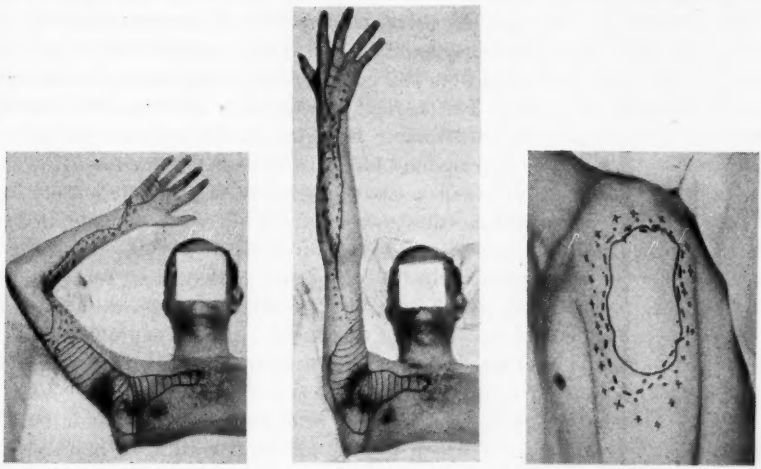
The pattern of sensory loss was also outlined in a patient (Pte C.) in which a tube pedicle had been raised on the lateral aspect of the abdominal wall. The pedicle had been constructed so that its long axis was approximately in the line of the ninth rib; it stretched beyond the costal margin to within 2 cm. of the midline, and was 4 cm. in width. Sensation in the skin was normal one-third of the way from its lateral attachment, beyond which there was a zone 1·5 cm. in width across the pedicle in which pain only could be aroused. Over the rest of the pedicle there was complete loss of all forms of sensation. A piece of skin approximately 1 cm. square was stained and removed from the zone in which pain only could be evoked. Only fine nerve fibres and superficial nerve nets giving rise to beaded endings were found on histological examination.

A further case was examined (Pte A.) who had a partial interruption of the ulnar nerve and transverse fractures of the radius and ulna in the lower third of the forearm. There was paralysis of the small muscles of the hand normally supplied by the ulnar nerve, and impaired sensibility over the usual area of distribution of its cutaneous branches. The patient stated that he could not feel the test objects so clearly or so strongly throughout the affected area as compared with the same area on the opposite hand. It was hoped that in skin from such a case the neurohistology might be somewhat simplified, as had occurred in the rabbit after partial section of the dorsal ear nerve. Skin was therefore stained and removed from the centre of the affected area on the dorsum of the hand. This piece of skin was 3 cm. square, but not of full thickness owing to the fact that it was shaved off by a razor. In a central area of the skin approximately 1·5 cm. square the staining was good and the outline of the cutaneous nerve plexus could be seen. In one limited field a single well-stained nerve fibre was seen among other unstained and presumably degenerated fibres of the cutaneous nerve plexus. After leaving the plexus this fibre was traced to its terminal ramifications in a nerve net situated immediately beneath the epithelium and covering an approximately circular area of 0·75 cm. in greatest diameter. There were no interlocking nerve nets in this area, presumably owing to the degeneration of the immediately adjacent fibres. Arising from the superficial nerve plexus, and at some distance from the position of origin of the fibre giving rise to the nerve net, was a single isolated Meissner's corpuscle borne upon a thick nerve fibre.

CLINICAL OBSERVATIONS

Nerve-root lesions. Two cases of nerve-root lesions were examined. The first case was that of a doctor (Dr M.) who had a laminectomy performed by Prof. Hugh Cairns. An 8 g. neurofibroma was removed from the lower cervical and upper dorsal region of the spinal canal. During the course of the operation

it was found necessary to divide the first right posterior thoracic root intrathecally before the tumour could be removed. Eight days after the operation, a sensory examination of the skin over the right upper extremity was carried out by Dr L. Guttmann. The area of sensory loss involved the skin over the whole medial half of the lower third of the arm, and skin over the medial half of the whole of the forearm. The areas of sensory loss for the four primary modalities of sensation were found to be almost completely superimposed in a concentric manner. The area of loss of touch sensibility was the smallest, being closely followed by that for pain. The area over which the sensation of



a *b* *c*

Text-fig. 7 *a* and *b*. Photographs showing pattern of sensory loss following section of the first posterior thoracic nerve root.

——— Touch; - - - - - Pain; 40° F. ..... Cold; 120° F. + + + + Warmth; Diminished sensibility.

Text-fig. 7 *c*. Photograph showing pattern of sensory loss following section of the fifth cervical posterior nerve root.

——— Touch; - - - - - Pain; 40° F. ..... Cold; 120° F. + + + + Warmth.

cold (40° F.) was lost was larger than that for pain, but smaller than that for warmth (120° F.). There was a slight diminution of sensibility to all forms of sensation over the second and third thoracic segments (Text-fig. 7 *a* and *b*).

The second case was that of a patient (T. A.) who had a laminectomy performed by Prof. Hugh Cairns for the removal of a 20 g. neurofibroma situated both extra- and intra-durally in the lower cervical region. It was again found necessary to section a posterior nerve root before the tumour could be removed, and in this instance the fifth left posterior cervical root was cut. Twelve days after the operation sensory examination of the skin was carried out by Dr L. Guttmann. The area of sensory loss involved the outer aspect of the upper half

of the arm. The pattern of sensory loss was similar to that in the case previously described (Text-fig. 7 *c*).

Nerve trunk lesions. A patient (Pte J. S.) was wounded by an explosive bullet in the left buttock. This resulted in a compound comminuted trans-trochanteric fracture of the neck of the left femur and injury to the sciatic nerve. Five months later the wound had healed and the patient had complete loss of sensation over the usual area of distribution of the common peroneal nerve. There was also complete paralysis of the muscles normally supplied by the nerve. The pattern of loss of the four primary modalities of sensation was similar to that usually found in such lesions. The area from which pain could not be aroused was the least extensive, being concentrically superimposed by an area in which touch sensibility had been lost. The areas from which cold and warmth could not be aroused were still larger and superimposed upon the area of loss of touch sensibility. The area of greatest loss was that for warmth sensibility. A sweating test (Guttmann, 1937) was carried out, and the area of anhydrosis was found to correspond closely to that of sensory loss. In only one position were points of sweat seen within the area of complete sensory loss (Text-fig. 8). There was hypoaesthesia over the whole area of skin supplied by the tibial nerve, and hyperalgesia along the outer side of the foot at the line of junction between the territories of the superficial peroneal and sural nerves. The patient was operated on by Prof. H. J. Seddon. The sciatic nerve was found to be embedded in a dense scar at the site of injury. A neurolysis showed that the tibial nerve was intact and appeared normal, whilst there was complete interruption of the common peroneal nerve.

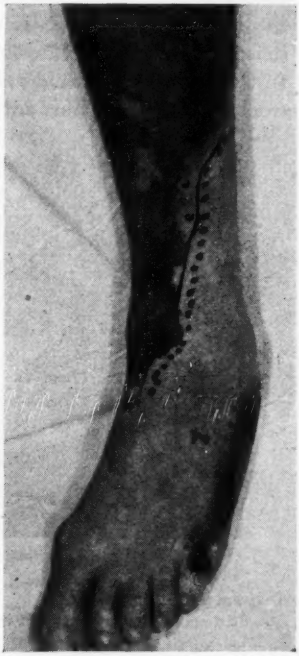
The pattern of sensory loss was found to be of a similar type in four cases of median nerve lesion, two of which were found at operation to be complete interruptions, in eight cases of ulnar nerve lesion, four of which were complete interruptions, and in eight cases of sciatic nerve lesion, three of which were found at operation to be completely interrupted.

Brachial plexus lesions. A patient (Pte B.) met with a motor-cycle accident in which he sustained what was probably a traction injury of the brachial plexus, involving the fifth, sixth and seventh cervical roots. There was motor loss which involved the triceps, deltoid, brachialis and supinator muscles. The pectoral muscles were weak. The areas of sensory loss comprised one over the lateral aspect of the upper part of the arm, and one on the back of the centre of the forearm. The areas of complete sensory loss were surrounded by areas of partial sensory loss. The patterns of loss of the four primary modalities of sensation were not concentrically superimposed. On the contrary, they overlapped each other excentrically. The area of loss of pain in this case was larger than that for touch in both positions, and the loss of both cold and warmth sensibility was still larger, the area of loss of warmth sensibility being the most extensive. The area of anhydrosis did not correspond in outline with any of the other areas of sensory loss.

Four other brachial plexus lesions were examined. The outlines of the

areas of sensory loss for pain and touch were found to cross each other at various points in all cases. In three cases of radial nerve lesions, known to be complete interruptions, the patterns of sensory loss were of a similar nature for all modalities of sensation.

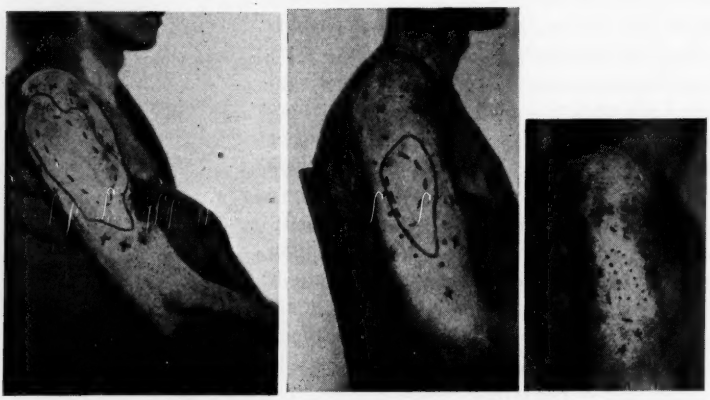
This series of cases serves to illustrate the relationship between the outlines of patterns of sensory loss for the four primary modalities commonly found in nerve root, plexus, and trunk lesions respectively. It is not suggested, however, that every case of nerve injury can be fitted into one of the three types of pattern described, for patterns of an intermediate type will be likely to occur after lesions at junctional regions between nerve plexuses and nerve roots and trunks respectively. The matter is further complicated by the fact that in some peripheral nerve trunks there is a tendency for the nerve fibres to continue their plexiform arrangement.



Text-fig. 8. Photograph showing area of sudomotor and sensory loss following a lesion of the common peroneal nerve. The arrow indicates points of sweat within the anaesthetic and analgesic area. ..... Pain; ———— Touch; 120° F. - - - - - Warmth; 40° F. ccccc Cold; + + + + Border of hyperalgesia.

Pattern of recovery of sensibility after complete peripheral nerve lesions. A patient (Dr K. S.) was examined who had had an acute inflammatory lesion of unknown aetiology involving the fifth and sixth cervical roots in the upper part of the lateral cord of the brachial plexus. The skin sensation was examined by Dr L. Guttmann 10 days after the onset of the lesion. There was loss of sensation over the upper and outer part of the arm. The area of analgesia was the smallest, being overlaid by an area of loss of touch, this in turn being overlaid by areas of loss of cold and warmth sensibility. A sweating test showed that the area of sensory loss was surrounded by an area of anhydrosis. The interval before recovery commenced (2 months) indicated that the lesion had completely interrupted the nerve fibres. During the course of recovery of sensation, the areas of sensory loss and anhydrosis were found to shrink in size. It may be emphasized that the value of the sweating test lies in the fact that it is objective in nature, and the close correspondence between the pattern of sudomotor and sensory loss in nerve lesions and during regeneration of nerve fibres has been noted previously (Guttmann, 1940). The pattern of sensory and sudomotor recovery in this case is illustrated by Text-fig. 9 *a–c*. The tests were performed at intervals of 3 and 5 months from the date of onset of the lesion.

A second patient (Sgt. A. H.) received a machine-gun bullet wound which completely severed the median nerve at the level of his right wrist. The nerve was sutured. Two months after the injury the areas of sensory and sudomotor loss were those typical of complete median nerve lesions at this level. The sweating test clearly indicated the overlap of fibres from the ulnar nerve into the median area which is commonly seen. One month later the areas of sensory loss and anhydrosis were found to have shrunk in size on the palm. In the thumb, index and middle fingers, the lines of recovery were advancing from the sides, and this advance was more marked at the base of the fingers



a *b* *c*

Text-fig. 9*a*. Photograph showing area of sensory loss after an inflammatory lesion involving the fifth and sixth cervical roots in the upper part of the lateral cord of the brachial plexus.
- - - - - Pain; ——— Touch; 120° F. + + + + Warmth.

Text-fig. 9*b*. Same case, 3 months later.
- - - - - Pain; ——— Touch; 40° F. ..... Cold; 120° F. + + + + Warmth.

Text-fig. 9*c*. Same case, 5 months later.
.....Pain; 40° F. - - - - - Cold; 120° F. + + + + Warmth.

and thumb. Two months later the only areas of complete sensory loss remaining were over the tip of the thumb, over part of the index finger, and over a small portion of the middle finger. The area of anhydrosis was rather larger (see Text-fig. 10 *a–c*).

These cases illustrate the fact that the recovery of cutaneous sensation may take the form of a circumferential shrinkage of the area of sensory loss rather than an advancing line of recovery commencing at the proximal border of the affected area.

Nerve-block experiments. Nerve-block experiments were carried out in order to determine whether there is any change in the limen of two-point discrimination or ability to localize stimuli in areas of partial sensory loss. The radial nerve was blocked by novocain 2 % and adrenalin 1 part in 20,000

in the lower third of the forearm in a normal subject (G. W.) experienced in the perception of sensory stimuli. The areas of loss of pain and touch sensibility were mapped out. The outline of the area of loss of pain sensibility was everywhere within that of the loss of touch sensibility. The borders of sensory loss between the ulnar nerve and the radial nerve on the dorsum of the hand, those between the median and radial nerves on the thenar eminence and thumb, and those in the web between the thumb and the forefinger, were not sharp. Two borders were outlined in these regions; the first by asking the subject to state when he first felt the test object, the second by asking him to state when the sensations aroused by the test object appeared normal. Both

a *b* *c*

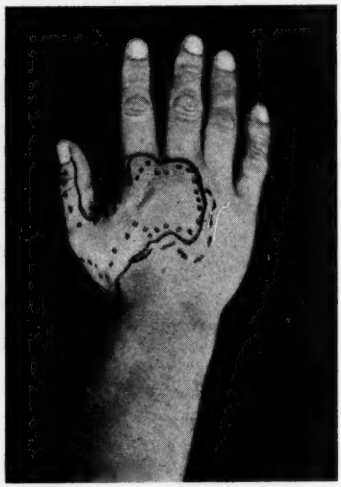
Text-fig. 10*a*. Shows the area of sensory sudomotor loss 2 months after complete median nerve lesion at the level of wrist.
——— Touch; ..... Pain.
Text-fig. 10*b*. Same case, 1 month later.
Text-fig. 10*c*. Same case, 2 months later.

borders were difficult to define, but the sensation within the intermediate zone was uniformly diminished. Further testing showed that two-point discrimination for touch and pain and the ability to appreciate the pattern of figures traced upon the skin were lost in the area, and localization was also impaired (Text-fig. 11). The radial nerve was subsequently blocked in the lower third of the arm and a similar area of sensory loss was found to have been produced; it was concluded from this second observation that the radial nerve had been completely interrupted by the anaesthetic.

CORRELATION BETWEEN THE HISTOLOGICAL AND CLINICAL FINDINGS

The pattern of innervation in the skin of the rabbit's ear is such that, in general, each unit area is evenly innervated by fibres approaching it from all directions. This is brought about by the scattering of the nerve bundles beneath the skin and the dichotomization of the nerve fibres as they pass

through the cutaneous nerve plexus to give rise to endings dispersed over approximately circular areas and evenly interlocked with endings derived from neighbouring fibres. There is histological evidence of the existence of a similar cutaneous nerve plexus in Man. The clinical finding that partial interruption of a main nerve trunk can give rise to a diminution of sensibility throughout its area of supply, and the finding with regard to the pattern of sensory and sudomotor recovery following certain nerve lesions, suggest that the cutaneous nerve trunks, on approaching the skin, rapidly disperse and ramify over wide areas before giving rise to a cutaneous nerve plexus, as in the case of the rabbit. For example, as the main trunk of the axillary nerve approaches the centre of the area of skin which it supplies, it appears that it then breaks up into bundles which travel throughout the area so as to approach their terminations from all parts of the periphery; that is to say, many of the fibres which innervate the central area of skin have the farthest distance to travel. In the case of the median nerve at the level of the wrist the same principle seems to be involved, but not in such an extensive form. The commonplace observation that small incisions through the skin are not accompanied by extensive areas of surrounding sensory loss also supports this conclusion.



Text-fig. 11. Photograph showing area of sensory loss following a radial nerve block in the lower third of the arm. ——— Touch; ..... Pain; - - - - - Boundary of the area within which there was hypoalgesia and hypoaesthesia.

The histological finding that, in areas of skin in which pain alone could be aroused, only fine nerve fibres giving rise to superficial nerve nets could be found, confirms a previous conclusion that such nerve nets subserve the sensation of pain (Woollard *et al.* 1940). It is further shown that the size of the superficial nerve nets varies in different parts of the body. On the back of the hand in Man, the net derived from a single fibre covers an approximately circular area which is 0·75 cm. in greatest diameter. This distance is approximately the same as the limen of two-point discrimination for pain in this region. The diameter of the nerve net from a single fibre in the skin of the pad of the thumb of the monkey is only 1·5 mm. in diameter. This approximates to, but is in fact rather less than, the limen of two-point discrimination in this region in Man.

In areas of partial sensory loss, such as those found between the radial, and ulnar and median nerves after blocking the radial nerve, the powers of

discrimination for two points and 'figure-writing' are lacking. Localization is also defective. Histologically, in an area of skin supplied by a partially interrupted nerve there are isolated endings such as single nerve nets and single Meissner's corpuscles, in contrast to the number of endings and close interlocking normally present.

It thus appears that the powers of localization and discrimination are dependent at least to some extent on the number of nerve fibres supplying unit areas; in other words, they certainly involve a factor of spatial summation.

The patterns of sensory loss following the nerve-root lesions described are similar to those described by Foerster (1936) and are such that the area from which pain cannot be aroused is larger than that from which touch cannot be aroused. It is suggested that this can be explained by the large amount of overlap normally present between the territories of adjacent nerve roots, and the smaller area supplied by the terminal ramifications of fibres subserving touch as compared with those subserving pain. A change in the pattern of sensory loss occurs in lesions extending through the brachial plexus until in the more peripheral interruptions of the median and ulnar nerves the area from which pain cannot be aroused is smaller than that from which touch cannot be evoked. The relatively small amount of overlap which occurs between these nerves compared with that between nerve roots, and the size and nature of the interlock of the terminal ramifications of fibres subserving pain and touch, presumably account for this change in pattern.

DISCUSSION

Evidence has been brought forward which indicates that as the cutaneous nerve fibres approach the skin they dichotomize and interlock with one another in the form of a cutaneous nerve plexus. This occurs in such diverse types as an elasmobranch fish, the rabbit, the monkey, and Man. The cutaneous nerve plexuses have been noted by a number of workers. Martinez (1934) mentions a cutaneous nerve plexus in connexion with free nerve endings, but it is not clear whether he is describing superficial nerve nets or the underlying cutaneous nerve plexus. Tamponi (1938) mentions that the nerve fibres supplying hair follicles are derived from a plexus of nerve fibres. Woollard (1936*b*) has described the cutaneous nerve plexus in the skin of the rabbit's ear. His general description accords well with the present observations, but the areas of skin examined were comparatively small.

In the elasmobranch fish only one system of cutaneous nerve fibres was observed, in the rabbit two systems of fibres, one set innervating the hairs and the other giving rise to superficially placed nerve nets. In the monkey and in Man there are still further sets of fibres and types of ending. There is evidence that in Man there are specific nerve fibres and endings which subserve the four primary modalities of sensation in the skin (Strughold & Karbe, 1925; Bazett, 1935; Woollard, 1935, 1936*a*; Woollard *et al.* 1940). There is no

histological evidence that any unit area of skin or cutaneous organ such as a hair is innervated by two sets of fibres or endings of different histological type, subserving each of the four primary modalities of sensation. On the contrary, evidence has now been brought forward that each unit area of skin or separate hair is innervated by more than one nerve fibre of the same type whose endings interlock with each other. In fact, each unit area of skin is innervated by a number of fibres which approach the area from all directions. Head's (1908) theory of protopathic and epicritic systems of nerve fibres was built on findings which were qualitative rather than quantitative in nature. His work was repeated by many other observers, the most detailed and elaborate studies being those of Trotter & Davies (1909), Boring (1916) and Lanier (1935). These workers approached the problem from a quantitative rather than a qualitative point of view, and disagreed with the findings of Head. Boring based a theory of cutaneous innervation on the dual innervation of each sensory spot, which fits in well with the present findings, but stated that the recovery of sensation did not follow the course of the nerve. He was, however, unaware of the nature of the pattern of cutaneous innervation. The findings of Trotter & Davies, Boring, Lanier, and other workers, that in general all forms of sensibility tend to reappear together after nerve division and suture, and that at first all returning sensation is diminished and then gradually approaches normal, can be interpreted in the light of the present histological observation. For it is now clear that each unit area of skin is supplied by nerve fibres approaching from more than one direction, the fibres having the farthest distance to travel presumably taking the longest time to re-establish functional connexions. The statement by Head that temperature of the epicritic type could only be aroused by large applicators at least the size of a sixpence is probably related to the histological finding that there are multiple endings which resemble Krause's end-bulbs borne upon two nerve fibres situated about 0·3 cm. apart in the skin in the proximity of a cold spot. The finding that the fibres giving rise to these end-bulbs also give rise to other branches within the cutaneous nerve plexus suggests that there are probably multiple groups of Krause's end-bulbs dispersed over relatively wide areas subserving each single cold spot.

It has been shown that in the normal skin there is a close interlocking between nerve endings, and that the size of the terminal ramifications of a single nerve fibre varies in different parts of the body. In the case of pain, the size of the ending is directly related to the limen of two-point discrimination. This confirms a suggestion made by Woollard *et al.* (1940) and a statement by von Frey (1899) that every nerve ending is distinct from every other nerve ending and that it is not possible to distinguish distances of sensation on the skin smaller than the distance between two nerve endings. The increase in the limen of two-point discrimination in areas of partial sensory loss, and the tendency towards poor localization, can be correlated with the histological findings of poverty of endings and the absence of interlock between the endings.

It has been shown by Weddell & Harpman (1940) that the nerve endings which probably subserve pain in the periosteum are in the nature of nerve nets, but that there is not the same intimate interlocking between the nets compared with that seen in the skin. Localization of pin-prick is also poor in periosteum and there is occasionally false reference.

The work of Kredel & Evans (1933) with regard to the recovery of sensation in various types of skin graft is interesting in the present connexion. They stated that the nerve fibres innervating pedicle grafts of full thickness whose polarity had been reversed during transplantation are re-innervated from above and to some extent from the sides, and that the direction of re-innervation bears no relation to the direction of the original nerve fibres in the flap. From this they concluded that the nerve fibres which re-innervate pedicle flaps do not follow the course of the old neurilemma sheaths, but form new pathways. The pattern of cutaneous innervation demonstrated in this paper indicates however that it is not necessary to draw such a conclusion.

In a previous paper (Woollard *et al.* 1940) it was suggested that the findings of Foerster (1925, 1927) that stimulation of the distal stump of a completely divided cutaneous nerve often gave rise to sensations travelling centrally in neighbouring cutaneous nerves was due to the passage of an impulse across closely interlocked endings. It is now clear that such an interpretation was unnecessary since the explanation must lie in the recurrent course occasionally taken by nerve fibres in nerve trunks. The findings of Foerster (1929) that stimulation of the distal end of a completely divided ulnar nerve at the wrist gave rise to pain referred to the fourth digit some months after the nerve section, and a similar observation in another case of a divided median nerve at the wrist, are explained on the basis of fibres passing from the median to the ulnar nerve across the communication which commonly occurs in this region, and running a recurrent course. In this connexion it can be stated that stimulation of the distal stumps of a number of completely divided nerves under local anaesthesia in Man by Prof. H. J. Seddon has so far elicited no sensation, but in no case has there been a known anatomical communication with neighbouring nerves in close proximity to the point of stimulation. With regard to the fact that nerve fibres have been seen to pursue a recurrent course within main nerve trunks, it is interesting to note that living nerve fibres growing into the tail of the frog tadpole have been seen to retrace their steps and run a recurrent course parallel to distally growing nerve fibres (Speidel, 1933).

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indebted to Dr L. Guttmann for his invaluable help with regard to the clinical investigations. The photomicrographs, Text-figs. 1 and 3, are the work of Mr W. Chesterman, whom I would like to thank.

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SUMMARY

1. The cutaneous nerve plexuses in skin from *Acanthias vulgaris*, the rabbit, the rhesus monkey and Man are constructed on a similar pattern.

2. Only one type of nerve ending was seen in skin from *Acanthias vulgaris*, two types were seen in skin from the ear of the rabbit, and multiple types in skin from the monkey and Man.

3. The pattern of cutaneous innervation in skin from the ear of the rabbit has been demonstrated. It is such that in general each unit area is evenly innervated by fibres approaching it from all directions.

4. A single nerve fibre in the dorsal nerve at the base of the ear of the rabbit breaks up into terminal ramifications which are distributed to approximately three hundred hair-follicle groups.

5. The terminal ramifications of a single nerve fibre distributed to hair follicles in the ear of the rabbit cover an approximately circular area of 1 cm. in greatest diameter.

6. Each hair-follicle group and each hair is innervated by at least two separate nerve fibres whose terminal ramifications are evenly interlocked with each other.

7. After section of approximately one quarter of the dorsal ear nerve in the rabbit, degenerating nerve fibres can be seen throughout the whole extent of the skin of the dorsum of the ear.

8. The size of a single superficial nerve net in skin from the pad of the thumb of the monkey covers an approximately circular area of 1·5 mm. in greatest diameter. It is closely interlocked with neighbouring nerve nets.

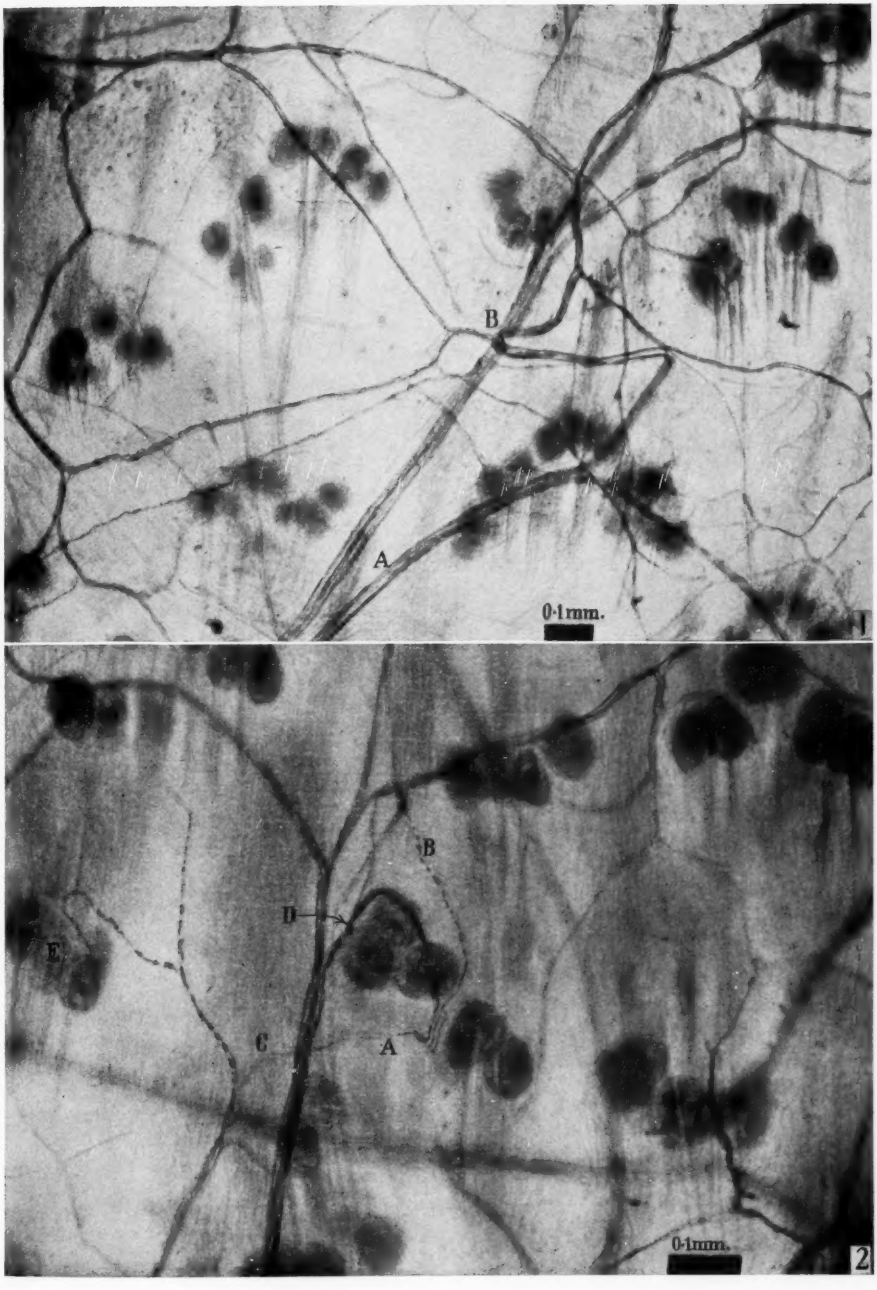
9. Multiple groups of Krause's end-bulbs are found beneath each cold spot in the skin of the human forearm. They are situated about 1 mm. from the skin surface and are borne on widely separated nerve fibres.

10. A single isolated superficial nerve net in a hypoalgesic area of skin from the dorsum of the hand in Man covers an approximately circular area of 0·75 cm. in greatest diameter. This distance corresponds to the limen of two-point discrimination for pain in a similar normal area.

11. The pattern of sensory loss after certain nerve root, nerve plexus and nerve trunk lesions in Man has been described.

12. The pattern of sensory recovery after certain complete nerve trunk interruptions has been described.

13. The clinical findings have been correlated with the histological findings and discussed.



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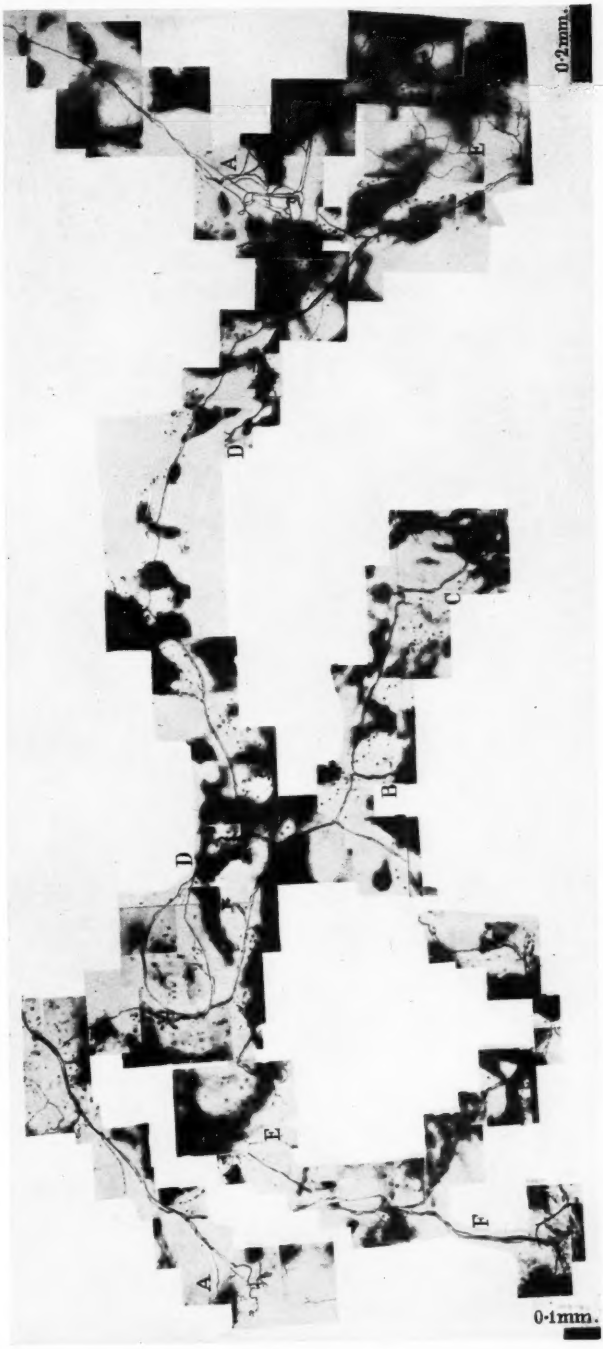

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EXPLANATION OF PLATES 1 AND 2

(All figures are photomicrographs from methylene blue preparations)

PLATE 1

Fig. 1. A subsidiary nerve bundle A is seen to be ending in the deep layer of the cutaneous nerve plexus. The nerve fibres are beginning to undergo dichotomization at B. Preparation from the skin of the dorsum of the ear of the rabbit.

Fig. 2. Shows a single hair being innervated by both normal and degenerating nerve fibres, 2 days after partial section of the dorsal nerve in the ear of the rabbit. A, hair; B, degenerating nerve fibre; C, normal nerve fibre; D, both normal and degenerating nerve fibres; E, hair-follicle group.

PLATE 2

This shows the neural topography in the neighbourhood of a cold spot in the skin from the forearm of a human subject. The photograph was retouched for the purpose of reproduction. A, groups of endings resembling Krause's end-bulbs; B, nerve fibres passing to Meissner's corpuscles; C, nerve fibres passing to capillary blood-vessels; D, nerve fibres passing to innervate hair follicles; E, superficial nerve nets; F, fibre proceeding from the subcutaneous tissue to join the cutaneous nerve plexus.

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THE EFFECT OF DENERVATION ON THE STRUCTURE AND HORMONAL RESPONSES OF THE MONKEY PROSTATE

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The influence of pelvic sympathetic and parasympathetic nerves on the secretion and structure of the accessory reproductive organs has been the subject of several investigations in recent years. Barrington (1913) demonstrated that in cats, guinea-pigs and rats both the hypogastric and pelvic parasympathetic nerves contain secretory fibres to the bulbo-urethral glands, and that only the former control the secretion of mucin. Continued stimulation of the hypogastrics leads to changes in the size, shape and staining reactions of the secretory cells, while prolonged stimulation of the pelvic nerves leads only to a diminution in size of the lumina of the acini and ducts. Further findings of Barrington were that no histological changes occur in the glands following unilateral division of either the hypogastric or pelvic nerves, and that the ganglion cells of the hypogastric fibres to the glands are situated distal to the inferior mesenteric ganglion.

Numerous investigations have shown that the hypogastric nerves, but not the pelvic parasympathetic nerves, also contain secretory fibres to the prostate (see Gruber, 1933, for review of earlier literature and Farrell, 1938, for a recent experimental study) and motor fibres to the seminal vesicle, but few studies have been made of the structural changes which follow denervation of these organs. Brouha (1938), summarizing a series of studies carried out in collaboration with Bacq, states that after removal of the inferior mesenteric ganglion, and to a lesser extent after extirpation of the lumbar sympathetic chains, the seminal vesicles, vasa deferentia and epididymes in the mature guinea-pig, and the prostate, prostatic utricle and vasa deferentia in the mature rabbit, become greatly distended with secretion. The same procedures produce no changes in the accessory organs of the immature animal, which develop normally at puberty. Gonadotrophic hormone, on the other hand, leads (indirectly) to greater growth of the seminal vesicles in the denervated mature animal than in the normal animal.

Changes in the prostate of mature dogs following division of the hypogastric nerves have been reported by Golub (1938), who found that the operation is first followed by distension of the alveoli, whose epithelium becomes flattened, and then, after about 20 days, by glandular involution. No changes were observed, however, when the nerves were divided in 2–4 months' old puppies. Four months after the operation the degree of alveolar differentiation was the

same as in litter-mate controls. The only other observation of which we know and which indicates that certain neural lesions may lead to involution of the accessory reproductive organs applies to the female rhesus monkey (Zuckerman, 1938 *a*); in this case it is possible that the change is indirectly due to changes in the ovaries.

In view of the observations reported by Golub, the following study was made of the effects of denervation on the prostate of rhesus monkeys (*Macaca mulatta*) and of the hormonal responses of the denervated organ. The study was limited by an unavoidable lack of experimental animals. Positive results were, however, obtained.

EXPERIMENTAL OBSERVATIONS

Four immature male rhesus monkeys, whose ages, as estimated from Schultz's tables for dentition and body weight (1933, 1935), ranged from 36 to 43 months, and one adolescent monkey estimated to have been 60 months old, were used in the experiment and were subjected to the procedures noted in Table 1.

Table 1

No.	Age months	Treatment
O.M. 467	43	Hypogastric nerves divided (day 1). Sacral nerves divided (day 56). Autopsy (day 209)
O.M. 468	41	Hypogastric nerves divided (day 1). Days 152–188 inclusive, 200 μg. oestrone daily. Autopsy (day 189)
O.M. 469	36	Sacral nerves divided (day 1). Days 151–187 inclusive 50 mg. testosterone propionate daily. Autopsy (day 188)
O.M. 470	40	Sacral nerves divided (day 1). Autopsy (day 208)
O.M. 471	60	Hypogastric nerves divided (day 1). Autopsy (day 213)

Hypogastric nerve resection. A midline laparotomy was performed, the nerves, which form discrete trunks in this species (Zuckerman, 1938 *b*), were freed from surrounding structures, and about 2 cm. of each were resected over the seventh lumbar vertebra. The wound was closed in the usual way.

Division of sacral nerves. To divide the sacral nerves, a midline dorsal incision was made, and the seventh lumbar and first sacral vertebrae were laminectomized. The dural sac was picked up on a hook, and all the sacral and coccygeal nerves divided immediately below the point of exit of the seventh lumbar nerves. The wound was then closed. In all cases healing was satisfactory and by first intention. This method of dividing the preganglionic pelvic parasympathetic fibres avoids surgical difficulties which are encountered in direct approaches to the 'nervi erigentes'.

In the case of those monkeys whose sacral nerves had been divided, micturition and defaecation were assisted daily for the first 2–3 weeks by gently pressing on the ventral abdominal wall. After this period, reflex evacuation of the bladder and rectum became established, although at autopsy the bladders were found to be considerably distended but not infected.

The hormones indicated in Table 1 were given by intramuscular injection in oil solution. The two animals which received the injections were given no treatment for 5 months following division of the hypogastric nerves in the one case and sacral nerves in the other, so as to allow a sufficient interval for any degenerative changes to occur in the prostates.

All five animals were autopsied about 200 days after the initial operation. The prostates were fixed in alcoholic Bouin's fluid, sectioned serially, and stained with Delafield's haematoxylin and eosin.

Numerous control prostates from animals of ages similar to those of the experimental monkeys were available for comparison.

Effect on structure of the prostate. The prostates of O.M. 467 and 470 suggest that section of neither sacral nerves alone, nor both sacral and hypogastric nerves together, has any effect upon the appearance of the immature glandular epithelium, all the characteristics of control specimens being retained. Thus, the glands are represented by a tubular system only, no alveoli having as yet differentiated. The number of glands per unit area appears unchanged. The epithelium consists of a single layer of cubical or short columnar cells, with large spheroidal nuclei and scanty cytoplasm; the lumen is characteristically small or absent. In the same way, the urethra, uterus masculinus and ejaculatory ducts are not different from the normal structures.

O.M. 471 was an adolescent animal, and its prostate is in a late stage of maturation. In spite of section of the hypogastric nerves, the differentiation of secretory alveoli has proceeded in typically normal fashion, and the epithelium, though as yet only partially active, is of the usual type; there is no sign of the involution reported by Golub in mature dogs subjected to the same procedure. Nothing abnormal is to be found in the uterus masculinus, urethra, collecting or ejaculatory ducts.

Responses to hormones. The responses of the prostate both to androgenic and to oestrogenic stimulation appear to be unaffected by nerve section. The fairly large doses of testosterone propionate (50 mg. per day for 36 days) given to O.M. 469 have resulted in marked glandular differentiation and to a considerable growth of the whole prostate (Fig. 1). The alveolar epithelium is actively secreting, and the alveoli and ducts are distended. The collecting ducts, uterus masculinus and ejaculatory ducts present no abnormal characters. The urethra, however, is abnormally distended almost throughout the prostate.

The prostate of O.M. 468, which was injected with oestrone, has also grown considerably (Fig. 1). The uterus masculinus shows the usual changes and is markedly enlarged, while its epithelium, like that of the crista urethralis, has undergone extensive hyperplasia and squamous 'metaplasia'. No alveolar differentiation has occurred, although in many places the lumen of the tubules is more open than in controls of corresponding age.

The responses of the prostates have been analysed further by measurement of the areas of the glandular, utricular and urethral regions in transverse

sections taken at the level of the middle of the uterus masculinus, following the method described by Zuckerman (1938 *c*). The dimensions are given in Table 2 and summarized diagrammatically in Fig. 1.

Table 2. *Dimensions of mid-utricular transverse section of prostate*

No.	Class	Whole organ actual sq. mm.	% glandular area	% utricular area	% urethral area
467	Hypogastric and sacral nerves divided	32·6	43·7	17·8	35·9
468	Hypogastric nerves divided: oestrone	89·3	50·1	28·0	18·8
469	Sacral nerves divided: test. prop.	150·0	71·6	5·1	17·8
470	Sacral nerves divided	33·1	56·9	12·7	29·2
471	Hypogastric nerves divided	97·3	77·0	5·7	17·3

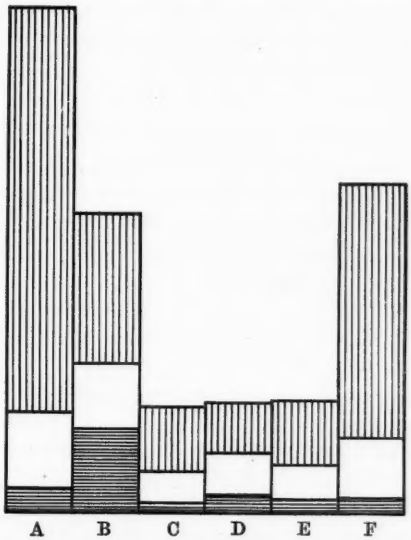

Fig. 1. The mean actual proportions, drawn to the same scale, of mid-utricular sections of the rhesus prostate after division of sacral and hypogastric nerves and after administration of male hormone and oestrone. Horizontally lined area = utricular bed; clear area = urethra; vertically lined area = glandular zone. A, sacral nerves divided; male hormone (O.M. 469). B, hypogastric nerves divided; oestrone (O.M. 468). C, mean of six controls of comparable age. D, hypogastric and sacral nerves divided (O.M. 467). E, sacral nerves divided (O.M. 470). F, hypogastric nerves divided (O.M. 471).

Fig. 1 shows that no significant differences exist between the relative proportions of mid-utricular sections of control prostates and those of O.M. 467 and 470, the immature monkeys whose sacral and hypogastric nerves had been divided. In the case of O.M. 471, the increased size of the glandular area,

associated with the animal's sexual maturation, accounts for the increased size of the whole prostate, and division of the hypogastric nerves has introduced no apparent complication into the normal course of development of the organ.

The administration of oestrone to O.M. 468, after section of the hypogastric nerves, has caused the usual significant increase in the size of the uterus masculinus and utricular bed (Zuckerman, 1938*c*, and Zuckerman & Sandys, 1939). Some increase has also occurred in the actual, but not the relative, extent of the glandular area.

The effect of testosterone propionate on the prostate of O.M. 469, after section of the sacral nerves, is again strictly comparable with its effect on the normal prostate (Zuckerman & Sandys, 1939). The significance of the urethral dilatation is not clear; similar dilatation has occasionally been observed in untreated animals.

No significant changes were observed, either in general appearance or in dimensions, in the capsular ganglia of the treated as compared with normal control animals. The ganglion cells were measured both by means of projections and from photomicrographs.

CONCLUSION AND SUMMARY

Division of the sacral parasympathetic fibres alone or of the sacral and hypogastric nerves together appears to have no effect on the histological structure of the immature rhesus prostate; in this respect the monkey behaves like the dog, guinea-pig and rabbit. On the other hand, division of the hypogastric nerves was without effect on the prostate of an almost mature monkey, a result which is not fully in accord with Golub's observations on dogs. Androgenic stimulation leads to rapid maturation of the prostate after division of the sacral nerves, while division of the hypogastric nerves has no effect on the responses of the prostate to oestrogenic stimulation.

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ANATOMICAL NOTE

A NOTE ON THE ACTIONS OF THE CRUCIATE LIGAMENTS OF THE KNEE JOINT

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Active and passive rotation of the knee joint are now well understood, both as regards causation and limitation. On the other hand, the final rotation at full extension associated with locking, a small movement of only about five degrees, has never been satisfactorily explained, though several authors, whose work has been admirably summarized by Fick (1911), have studied the problem with great care. In the present note some aspects of ordinary rotation are considered as a preliminary to the study of locking (though most of the points mentioned are already well known), and then an attempt is made to find the mechanical cause of the final rotation.

A knee joint with all connexions between the femur and the neighbouring bones cut except the collateral and cruciate ligaments behaves as an intact joint, and shows normal locking movements; presence or absence of the semilunar cartilages makes little difference. The range of movement corresponds closely to that of passive movements in the living subject, studied by Ross (1932) and others, though voluntary movements of rotation are often more limited. Doubtless in life the ligaments are protected from undue strain by muscular reflexes, but all authors are agreed on the importance of the ligaments in determining the range of the movements.

The position of the femur relative to the tibia at any particular degree of flexion is decided partly by the intercondylar eminence of the tibia which articulates with the intercondylar fossa of the femur so that no lateral displacements are allowed, and partly by the cruciate ligaments. These are arranged so that in all positions of the joint forward movement of the femur on the tibia is prevented by the posterior cruciate and backward movement by the anterior cruciate. In the flexed knee, however, the ligaments are relatively slack, so that some small degree of antero-posterior movement of the tibia is possible as a passive movement, both in the living subject and in the cadaver. Now, since their actions are reciprocal, the tensions in the two ligaments will be about equal (Fig. 1 B). For, should the tension in one ligament be great, say in the anterior ligament, the femur will be pulled forwards on the tibia until the tension in the posterior ligament is increased so that it exerts a backward pull sufficient to balance the forward pull of the anterior ligament. This tendency to equality may be modified by variations in the obliquity of the attachments of the two ligaments with different positions of the joint (Fig. 1 A, C), and by muscular and gravitational forces, but apart from these if one ligament is taut the other must also be taut.

The attachments of the cruciate ligaments to the tibia lie directly anterior and posterior to each other, while the anterior ligament is attached to the femur lateral to the posterior. So, when the femur is rotated laterally on the tibia, both ligaments are tightened, being 'wound up' round each other, and they limit rotation in this direction. On the other hand in medial rotation of the femur the ligaments are 'unwound' and slackened, so that this movement is limited entirely by the collateral ligaments.

These interpretations can be checked on the cadaver. Cutting both collateral ligaments makes no difference to the amount of lateral rotation of the femur, but the range of medial rotation is greatly increased, and as the ligaments become 'unwound'

the tibia drops away from close contact with the femur. Cutting the cruciate ligaments slightly increases the range of lateral rotation, which is now checked by the collateral ligaments, but medial rotation is not affected. Cutting even one of the cruciate ligaments increases antero-posterior mobility, and this is shown clearly in patients whose ligaments have been torn.

The arrangement of the cruciate ligaments also determines the rolling of the femur on the tibia in the later stages of extension. As the knee becomes more extended the femoral attachment of the anterior ligament is carried away from the tibia, so that if the femur did not roll forward on the tibia the ligament would become stretched. At the same time the attachment of the posterior ligament moves nearer the tibia, and this ligament would be loosened. Actually the femur rolls forward so that the antero-posterior forces exerted by the two cruciate ligaments are equalized. According to Fick (following Fischer), the rolling movement can be detected in the living subject only in the last twenty degrees of extension.

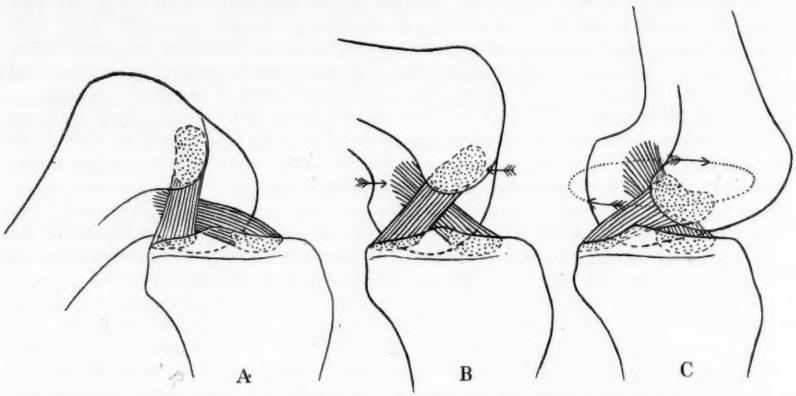

Fig. 1. Diagrams of the left knee joint with the medial condyle of the femur partly cut away, to show the cruciate ligaments. A, in full flexion. B, in mid flexion: the arrows indicate the antero-posterior forces exerted by the cruciate ligaments. C, in extension: the circle indicates the rotation produced by the tightening of the cruciate ligaments.

The femoral condyles are more rounded posteriorly and more flattened anteriorly, a point studied in detail by Higgins (1896) and others. The collateral ligaments are attached near the centre of curvature of the posterior parts of the condyles, so that as the anterior parts come into articulation with the tibia these attachments are carried away from the tibia and the ligaments are stretched. The change in curvature also affects the cruciate ligaments, for the point lying midway between their attachments to the femur is equally carried away from the tibia. Further, since these are fairly taut during the earlier stages of extension, while the collateral ligaments are relatively slack to allow for rotation, the cruciate ligaments will be the first actually to become taut. The two ligaments pulling on the femoral condyles in opposite directions now induce the medial rotation of the femur associated with locking. This rotation however cannot proceed far, for the collateral ligaments, already tightened during extension, soon bring it to a stop. The joint is now fully locked, with the cruciate ligaments tending to turn the femur medially and the collateral ligaments laterally, and all four ligaments are taut.

Checking these points on the cadaver, all four ligaments are in fact found to be taut when the knee is locked. Fick notes this point, though he subdivides each

cruciate ligament into two parts, only one of which is taut. Most other authors either state definitely that the anterior ligament is taut while the posterior is slack, or imply that this is so by explaining the rotation in locking as a rotation about a taut anterior ligament. In the preparation with only the four ligaments connecting the tibia and femur cutting of either cruciate ligament gives some hyperextension. If both cruciate ligaments are cut the joint still locks in hyperextension, due to the action of the collateral ligaments, but there is no rotation. Cutting the collateral ligaments allows great hyperextension with extreme medial rotation of the femur, due to the unchecked action of the cruciate ligaments, such as would be expected if the suggestions here made as to the causes of rotation are correct. On the other hand, in a formalin-fixed joint in which the posterior part of the capsule and the semilunar cartilages are left intact, the movements may be little influenced by cutting any or all of the ligaments. This may at first sight seem to invalidate the claim here put forward that the two cruciate ligaments acting together produce a couple that rotates the femur. But, clearly, when the joint has been fixed in the fully extended position, and the parts around it have been rendered hard and inelastic, the knee will, on extension, take up again the position in which it was originally fixed. Thus a preparation in which the cruciate ligaments have been cut without interference with the posterior parts of the capsule still shows the rotation at locking, though this rotation has now little force, but if the capsule and semilunar cartilages are now cut the rotation is lost. The capsule, tending to check the movement of the medial condyle before that of the lateral, may be an accessory factor inducing rotation, but can be of no great importance.

Again, the difference in the shape of the two condyles may also be an additional factor in the rotation, but as Fick points out, it cannot be a fundamental cause. A model made with strings representing the collateral and cruciate ligaments, but with the two condyles exactly similar in shape and size, carries out normal locking movements.

The mechanical principles underlying the tendency to rotation produced by two taut cords arranged as are the cruciate ligaments are well known to physicists as those of 'bifilar suspension'. The axis of the rotation is difficult to define as the rotation itself is so limited and various opinions as to its exact location have been held. The amount of the torque exerted by the two ligaments is however independent of the position of this axis.

Thus it appears that the primary function of the cruciate ligaments is limitation of antero-posterior movements, and that they are crossed for this purpose. The rotation of locking is a consequence of this crossing, and is a small movement of no particular functional significance. In fact, the knee joints of several animals, for instance the squirrel, show the rotation of locking just as in man, though such animals never stand on a locked knee.

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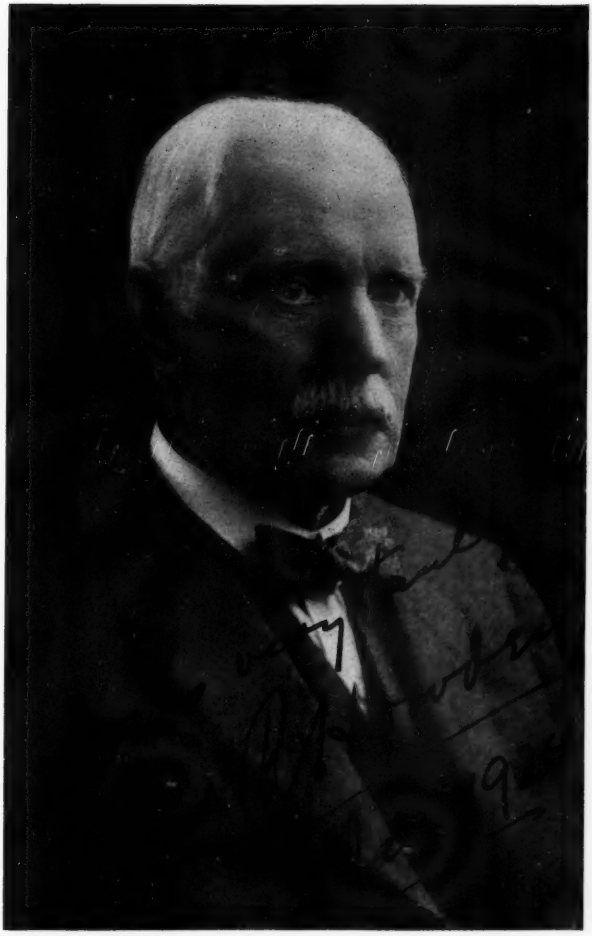
IN MEMORIAM

ROBERT HOWDEN, M.A., D.Sc. Dunelm, M.B., C.M., LL.D. Edin., F.R.S.E.

The death of Emeritus Professor Robert Howden at Crieff, Perthshire, on 7 December 1940, removes not only one of the great anatomists of the Edinburgh School and a very old member of the Anatomical Society, but also a figure noteworthy in the history of the University of Durham College of Medicine, Newcastle-upon-Tyne. He was born in Braco in Perthshire in 1856, and after qualifying at Edinburgh University in 1884 he became Demonstrator under Sir William Turner. He held this position for five years and for a short while worked with His at Leipzig. At this time the Edinburgh School was providing most of the anatomical teachers for the British Empire. Howden was one of these and he was also one of the number of Turner's pupils and assistants who later dedicated to their master Cunningham's *Text-Book of Anatomy*, a work which is to-day a monument to him in whose name it was dedicated as well as to those who were the original contributors.

When Howden left Edinburgh he took up an appointment in Newcastle where he was appointed Lecturer in Anatomy in the College of Medicine in 1887, and four years later became its first Professor of Anatomy. He held this position until his retirement in 1927. His appointment coincided with the time when the rapid increase in the size of the School necessitated its removal from the small and inconvenient buildings in Orchard Street to the much more commodious premises in Northumberland Road, which were to be the home of the College for fifty years and which were only vacated in 1938. Coming to the School when it was really taking on functions of vastly increased importance as a College in the modern use of the term, Howden found plenty of work to occupy his time and energy. He commenced with the development of his own Department and enriched it with many beautiful mounted specimens of his own dissection which replaced the old dried and varnished specimens previously in use. He also provided for the Department a series of wall diagrams, many of them his own handiwork, which were a great adjunct to his teaching; some of these are in use to this day. Anyone familiar with the labour which this type of work entails will appreciate how much time he must have devoted to his department in these early days.

His undoubted teaching ability soon expressed itself and contributed largely to the growth and success of the School, then at a crucial stage of its development. Those who knew him at this time recall the vigour and energy which he put into his work not only in the dissecting room, but also in his lectures and demonstrations. They recall too his great artistic ability on the



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blackboard and the care with which his lectures were illustrated by diagrams and beautifully dissected specimens. He was always insistent on the value of the freshly dissected part in teaching and frequently required his Demonstrators to prepare dissections, perhaps at short notice, for use in his lectures or to clear up some doubtful point.

During those early years in Newcastle, Howden was the only full-time member of the staff, and being thus always available in the building, management and administrative duties were heaped upon him. As a result, in due course he became Secretary, Dean of the Faculty of Medicine and Registrar, in addition to maintaining his professorial duties. For many years he bore ably the greater part of the burden of the administrative work of the College; he looked after its daily life and management and guided its policy. Anyone who was not associated with the College during this time of Howden's greatest activity will fail to appreciate the important part he played in the life of the College or the way in which both staff and students came to rely upon his wisdom, guidance and sympathy. Through the generations of students who came under his care he exercised a profound influence on the medical profession in the north of England. He represented the University of Durham on the General Medical Council from 1918 to 1928, and he was Examiner in Anatomy for the Universities of Edinburgh, Oxford, Cambridge, Liverpool and Birmingham and for the Conjoint Board of the Royal College of Physicians and Surgeons.

Though so much occupied with administrative and teaching duties, Howden still found time to advance the study of his subject. Between the years 1886 and 1897 he made several communications to the Anatomical Society of Great Britain and Ireland, and his work for, and interest in, the Society were recognized when he was elected its President in 1914. During his presidency the *Journal of Anatomy and Physiology* became the *Journal of Anatomy*, and the official organ of the Anatomical Society of Great Britain and Ireland. He also edited nine successive editions of Gray's *Anatomy*, two of them in collaboration with T. Pickering Pick, and through this medium his influence spread far beyond the confines of the School in which he worked. As editor, he maintained that meticulous attention to detail both in the text and the illustrations which did so much to enhance the prestige of this text-book.

The Universities of both Edinburgh and Durham recognized his exceptional merit and services to University life by conferring Honorary Degrees upon him.

There is no doubt that Howden's name will be most remembered by those whose privilege it was to know him, not so much for his part in the advancement of anatomy, to which he was no mean contributor, or for his years of devoted service to the University of Durham, but for his spacious and lovable personality in which honesty, kindliness and generosity were outstanding and which overshadowed mere academic distinctions or attainments. He was a great man and acknowledged as such by those who worked with him and to

whom his memory is a treasured possession. To his juniors he was invariably polite and courteous. He was always a stimulus to them and was never too busy to listen to their problems or to help them by advice and encouragement. He was a charming companion and enjoyed social occasions, and in the pursuit of his favourite recreations of golf, fishing and curling he found many friends.

After his retirement his health, which had been far from good for some years, failed still further and he had to face many surgical ordeals. He met these with a stoicism characteristic of the brave heart and undaunted spirit which had been so evident during his active life.

R. B. G.

MATTHEW YOUNG, 1884–1940

MATTHEW YOUNG died at his home in Esher on 25 May 1940, at the age of 55, after a protracted illness borne with characteristic patience and fortitude. His passing has bereft the science of human anatomy of a gifted biometrician, and has left his many friends deeply conscious of their loss.

Young not only possessed a sound and practical knowledge of those aspects of human anatomy which are the common ground of teaching and investigation in every medical school, but he had a flair for mathematics which, dating from the days of his boyhood at Kilmarnock Academy, developed and ripened with the passing years until it bore rich fruit in those biometrical and statistical studies with which his name will always be associated.

After a distinguished undergraduate career at Glasgow University, followed by a short period of general practice in Yorkshire, he began his anatomical career in 1908 as junior demonstrator under John Cleland, and subsequently acted as senior demonstrator for six years under Thomas Hastie Bryce.

The happy experiences and inspiration of those early days in the famous Glasgow school were often gratefully referred to by Young in later life.

In 1915 his Alma Mater awarded him the degree of M.D., with honours and a gold medal, and also the much coveted prize for anatomical research, the John Struthers gold medal. His thesis, *A Contribution to the Study of the Scottish Skull*, is a model of clear thinking and painstaking accuracy and was published by the Royal Society of Edinburgh in 1916.

Young was now launched upon a career which but for the momentous events of 1914–19 would have brought him naturally and inevitably to the occupancy of an anatomical chair, a life-long desire which he never concealed from his nearest friends.

During the years of war, however, those dreams were laid aside, and Young rendered a signal service to his country which, though publicly acknowledged at the time, is perhaps insufficiently known to a younger generation.

In association with his friend and chief, Dr John Brownlee, also an alumnus of Glasgow University, there was carried through in response to a clamant need that *magnum opus*, if ever there was one, of collecting, collating, and card-indexing the complete medical histories of all cases arriving in this country from war hospitals abroad. This involved tabulating, analysing and comparing the results obtained with the various forms of treatment and was a work of supreme value. It was a colossal task, the technical side of which only an indefatigable statistician of the first class could have carried through to success. The result represents the unstinted labour of seven years and remains as a monument of accuracy and achievement for all time to those who follow after.

The completion of this task saw Matthew Young free to return to his first love, human anatomy, but biometrical and statistical research still claimed

him and, fortunately for us, did not let him go. He found a new home in the Institute of Anatomy at University College, London, where he was appointed lecturer in anatomy under Sir Grafton Elliot Smith and Professor J. P. Hill, but by a most happy and farseeing arrangement Young continued to be a member of the scientific staff of the Medical Research Council. In those years of dual responsibility he frequently expressed to his friends his appreciation of these relationships which gave him the opportunities he delighted in for teaching and research.

His output of published work was a steady and well-regulated stream; never a year passed without something of real scientific value emanating from that room in Gower Street where he worked so quietly and unobtrusively.

From 1915 when he had made his debut with observations on the Scottish Skull to 1940 when, in spite of illness, his genius for biometrics and statistical interpretation never flagged, his work bore the hall-marks of the true scientist, exactitude and unremitting toil.

The remembrance of his gentle courtesy and unflagging industry; his unselfish service of those who needed him and who never appealed in vain; his steadfast and uncomplaining courage as he fought his battle with the disease which claimed his body but never could subdue his spirit; and above all, the example of his unswerving loyalty to accuracy and truth, are some of the things which, in addition to his published works, he has bequeathed to us for our permanent possession.

J. K.

A PRESOMITE HUMAN EMBRYO WITH A NEURENTERIC CANAL (EMBRYO R.S.)

By P. N. B. ODGERS
Department of Human Anatomy, University of Oxford

The paraffin block containing the embryo described in this communication was given to me by Dr Robb Smith, Pathologist to the Radcliffe Infirmary, who was also good enough to furnish me with the following information about its origin. He found it in a uterus which had been removed by hysterectomy the same day from a woman, aged 40, and which contained several intramural fibroids undergoing red degeneration. The whole of the endometrium was thickened, and a small cyst was noticed in the lower posterior quadrant; this was excised with the corresponding portion of the uterine wall. The excised specimen was fixed in 5 % formol-saline and embedded in paraffin. When a few sections had been cut and stained, Dr Robb Smith discovered the presence of a blastocyst; he therefore handed the rest of the block to me.

The menstrual history was as follows. The last period began on 5 October, and the period which was due on 2 November was missed. The uterus was removed on 15 November. From these data the minimal age of the embryo must have been 22 days.

The block was cut into serial sections 10 μ in thickness, and these were stained with haematoxylin and eosin. They were found to contain the complete embryonal rudiment. Each section was photographed at a magnification of 150, and a reconstruction model of the embryonal disc was made at this magnification by the blotting paper and beeswax method. This latter has been used by myself for many years in this Department, and I consider that it is unrivalled for its accuracy and durability. From the reconstruction the sections were found not to be truly transverse to the longitudinal axis of the embryonal plate. The angle which the plane of section makes with that transverse to the embryonal axis increases in the cranial half of the disc. It is 10° midway along the primitive streak, 15° at the level of the neurenteric canal, while it is 40° half-way along the chorda plate and almost 80° in the prochordal area. These differences are partly due to the angle which the chorda plate makes with the primitive streak at the site of the neurenteric canal (see below). Nor are the sections accurately vertical: they are cut with an obliquity which is most marked in the cranial end of the embryonal plate.

From the account of it which follows, the embryo will be seen to fall into the group to which the following four previously described specimens belong:

Frassi (1907, 1908), length 1·17 mm.
V. Spee, Embryo Gle (1889, 1896), length 1·5 mm.
Triepel, Embryo Dy (1917), length 1·6 mm.
Boerner-Schwarzacher (1923), length 0·47 mm.

While all of these are very similar to the present specimen in their size (with the exception of the Boerner-Schwarzacher embryo) and in the appearances they present, the Frassi embryo seems to be the most like, and many of his drawings might well illustrate, my description. The only substantial differences between the two are the length and shape of the embryonal disc, the Frassi disc being both shorter (1·17 mm.) and narrower (0·6 mm.) than mine. Hill & Florian (1931) have drawn attention to the two varieties of embryonal shield, the one small and comparatively narrow, the other large and comparatively broad. My embryo, R.S., evidently belongs to the latter type.

DESCRIPTION OF EMBRYO

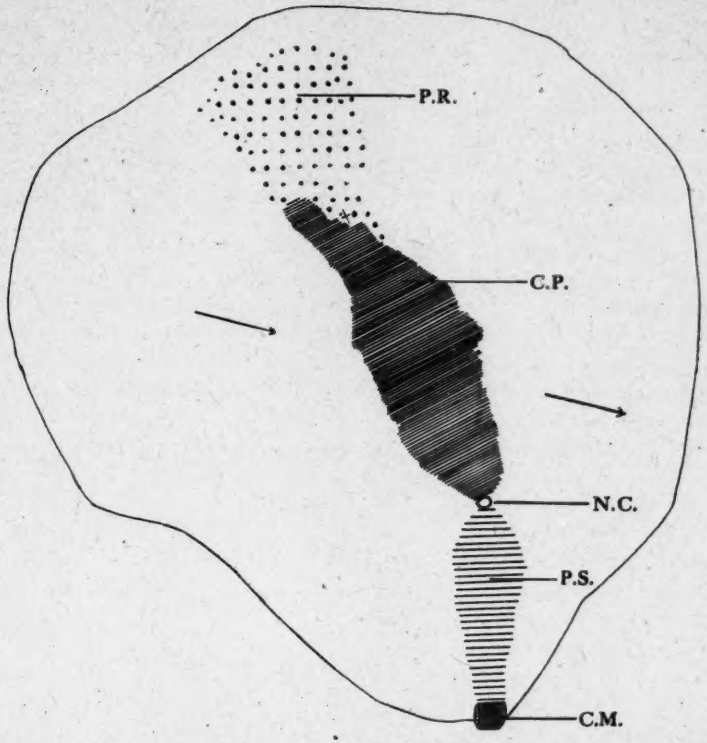
The germ disc (Pl. 1, fig. 1 and Text-fig. 1). The embryonal area is roughly triangular in shape and its greatest breadth is not far short of its axial length. The following measurements of the disc and of the axial structures were made with a flexible tape measure on the reconstruction model (× 150) and reduced:

Length of disc from the cranial amniotic reflexion to the caudal end of the primitive streak	1·5 mm.
Greatest breadth of disc	1·36 mm.
Length of primitive streak	0·4 mm.
Length of chorda plate	0·7 mm.
Length of prochordal plate	0·29 mm.
Length of area in front of prochordal plate	0·08 mm.

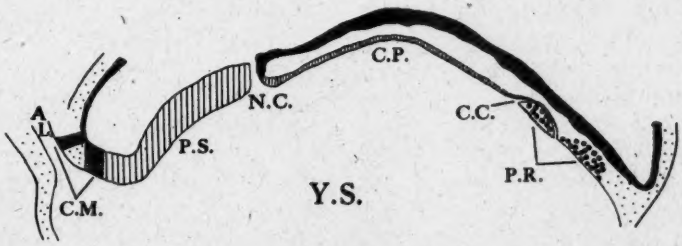
The embryonal disc exhibits a marked dorsal convexity in either plane, the greatest height of this being about half-way along the chorda plate where it rises to 0·37 mm. above the base line. While in the transverse plane this convexity forms an even curve, along the cranio-caudal axis of the disc (Text-fig. 2) it is definitely angulated midway along the chorda plate. In Triepel's embryo this angulation, which occurs at about the same situation, is increased to a right angle.

There is no bend at the site of the neurenteric canal, as is seen in V. Spee's embryo Gle in which the streak area makes almost a right angle with the cranial portion of the plate at this point.

A dorsal view of the reconstruction model (Pl. 1, fig. 1) shows at the junction of the caudal third with the anterior two-thirds the opening of the neurenteric canal, while behind this is the primitive groove. Immediately in front of the neurenteric orifice is a small projection which may be the remains of Hensen's node, and in front of this again the general dorsal convexity is broken for a short distance by the commencing medullary groove. Two further points may be observed in Pl. 1, fig. 1 and in Text-fig. 1, in both of which the different portions of the embryonal axis are mapped out. In the first place there is considerable asymmetry of the disc, so that roughly three-fifths to two-thirds of it, varying between these limits at different levels, lie to the left side of the



Text-fig. 1. A projection of the dorsal aspect of the embryonal shield on the flat (× 75), drawn to scale. *C.M.* cloacal membrane. *P.S.* primitive streak. *N.C.* neurenteric canal. *C.P.* chorda plate. *P.R.* (with coarse dots) prochordal plate. The † at the cranial end of the chorda plate indicates the site of the remains of the chorda canal. The arrows indicate the plane of section.



Text-fig. 2. A vertical longitudinal section through the embryonal axis (× 75) drawn diagrammatically to scale and idealized to permit a representation of all the axial structures in the same section. *AL.* allantois. *C.M.* cloacal membrane. *P.S.* primitive streak. *N.C.* neurenteric canal. *C.P.* chorda plate. *P.R.* prochordal plate. *C.C.* remains of chorda canal. *Y.S.* yolk sac.

embryonal axis when viewed from above. Secondly, it is apparent that the chorda plate and the primitive streak are not in the same straight line. The chorda plate is inclined to the left and makes an angle of 25° with the line of the primitive streak at the site of the neurenteric canal. This deviation is figured as being more marked still in the Boerner-Schwarzacher specimen and may be readily explained, I think, by the attachment of the massive connecting stalk to the caudal end of the plate. While the streak area is fixed in this way, the open neurenteric canal very possibly allows a certain amount of mobility to the anterior portion of the disc.

The primitive streak. The measurements of the extent of the primitive streak and the chorda plate (0·4 and 0·7 mm.) show that the former is of greater length than would be expected at this stage in comparison with most of the other embryos of about this age. In the Frassi embryo about half of the axial formation is represented by the streak so that the latter is even more extensive than in mine. In Triepel's embryo, however, only one-seventh of the embryonal area is stated to be caudal to the neurenteric canal, while in Rossenbeck's embryo Peh-Hochstetter (1923), which is younger than mine, the proportion between streak and head process is 2 to 5 and in Ludwig's specimen (1928), which is slightly older than mine, it is almost 1 to 5. Both these latter measurements are taken from Florian's (1934) *Schema der Entwicklung der Axialgebilde des menschlichen Embryos*, in which the gradual diminution of the streak area in comparison with the steady elongation of the head process is graphically shown. Into such a scheme as this my embryo hardly fits.

Pl. 1, fig. 2 is a microphotograph of a typical section of the streak area. A small point to which attention may be directed is the condition here of the entoderm. While it generally takes no part in the fusion of the germ layers and forms a distinct layer throughout its extent, the cells composing it are crowded together and give the appearance of sharing in the cellular activity of the neighbourhood. In more than one place, moreover, particularly in the caudal end of the streak, there are connexions between it and the overlying mesoderm, which almost suggest that it may even still be contributing to the formation of the latter. In earlier embryos fusion of the entoderm and of the mesoderm of the primitive streak has been frequently observed (Florian, 1932–3).

The neurenteric canal (Pl. 1, fig. 3). This is seen to be patent in two sections and is oval in shape, its longer axis being in the antero-posterior plane. The lips of the canal shelve more gradually on its ventral than they do on its dorsal aspect, this being particularly marked in the cranial lip. Filling the lumen is an amorphous coagulum stained with eosin. The canal is a true neurenteric canal, being a vertical connexion between the amniotic and yolk sac cavities. Grosser (1924) insisted that the term should be confined to such a channel and should not be used synonymously with the chorda canal. The Boerner-Schwarzacher embryo showed a neurenteric canal with a larger lumen than mine: it was similarly oval in shape and measured $40 \times 30\,\mu$ in its longitudinal and transverse diameters respectively. Sternberg (1927) suggested that such a widely open

canal perpendicular to the disc was due to post-mortem changes and was only found in abortions (e.g. V. Spee's Embryo Gle and Triepel's Embryo Dy), or in cases such as the Boerner-Schwarzacher embryo, in which 40 hr. had elapsed post-mortem before the specimen was found in the uterine wall, and he supposed, therefore, that it might not be truly characteristic of human embryos. He contrasted with these the oblique canals of the Frassi and M'Intyre (Bryce, 1925) specimens, but in the former of these the obliquity, as shown in the original drawings, is very slight, and Frassi stated, moreover, that he could not determine its patency owing to a damaged section. Again, the M'Intyre embryo is not comparable with the group under consideration, as this contains the remains of a chorda canal rather than the true neurenteric canal of Grosser's definition. On the other hand, both my own and Ludwig's Da I embryos were obtained by operation, and while the neurenteric canal in the latter had a narrower lumen it was, like mine, perpendicular to the embryonal disc.

The chorda plate (Pl. 1, fig. 4). This forms a flattened plate of varying thickness composed of round cells. It is thicker at its centre than at its periphery, one to three layers of cells being counted in the different portions in any one section. The plate is interpolated in the entoderm with which its edges are continuous, but in no case does any entoderm clothe its ventral aspect. Nor can I see in any of my sections that the chorda plate is recessed and bounded by distinct lips of the entoderm as Bryce (1925) described in the M'Intyre embryo (the bourrelets lécitho-entériques of Van Beneden). In some sections the plate is in direct continuity at its periphery with the streak mesoderm, and exceptionally the latter just overlaps the margin of the plate to separate it from the overlying ectoderm. For the most part, however, they are distinct from each other so that there is little, if any, evidence in this embryo that the chorda plate contributes anything to the formation of the mesoderm. One cannot but be impressed by the breadth of the plate. Allowing for the obliquity of the sections in this specimen, it seems impossible to think of it as being merely the remains of the dorsal wall of the chorda canal. As far as I know, Triepel is the only author who states that the plate increases in circumference after the disruption of the canal, but one must believe that this is the case. By the same token, as Bryce and others have insisted, the breadth suggests that it must yield something more than simply the notochordal rudiment, i.e. that it probably helps to form the entoderm of the digestive tract.

Caudally the plate becomes continuous with the ectoderm lining the cranial lip of the neurenteric canal. I cannot make out any certain evidence of either chorda plate or of the remains of the head process posterior to the neurenteric canal. Ludwig (1928) described in his embryo Da I twin anlagen of the plate joined in the midline, which he observed in the first section caudal to this opening.

In the sixth, seventh and eighth sections from the cranial end of the chorda plate can be seen a small collection of cells similar to those of the plate, which

forms a short oval mass lying between its centre and the ectoderm and contiguous with both of these (Pl. 1, fig. 5). This appears to be solid throughout its extent. Does this represent a premature attempt at the formation of a notochord? Triepel suggested that in his embryo Dy there was possibly an indication of a commencing notochord, but this consisted of a small narrow dorsally directed evagination of the chorda plate close to its junction with the entoderm and therefore not median in position, which occurred in some sections not far cranial to the neurenteric canal. If these appearances in my own embryo or in the embryo Dy have any such significance, they must be regarded as premature efforts. The earliest true evidence of the formation of a notochord from a chorda plate that has been recorded, so far as I am aware, is furnished by Corner's (1929) embryo with ten somites.

The prochordal plate (Pl. 2, fig. 6). The thickening of the entoderm with its characteristic chromatophilic granules (Bonnet, 1901) is seen to extend for 0·29 mm. cranial to the commencement of the chorda plate (Pl. 1, fig. 1 and Text-figs. 1, 2). As the latter appears to be more extensive on its left side, the prochordal plate overlaps it on the right for 0·13 mm. Hill & Florian (1931) described it in the embryo Dobbin as being prolonged backwards for 70 μ on either side of the head process. In my embryo the prochordal plate does not reach the cranial margin of the embryonal disc but is separated from it by a region 0·13 mm. in extent in which the entoderm is unaltered. The plate shows in four sections some irregular cavitation, and in its caudal portion is traversed in two sections by what appears to be a short canal. This channel, which is seen to be obliquely sectioned, may or may not be patent, but can be observed in Pl. 2, fig. 8 to be continuous by its dorsal wall with the chorda plate, while in the next section cranial to this (Pl. 2, fig. 7) it opens ventrally and its dorsal wall becomes continuous with the entoderm, the whole canal passing dorsal to the prochordal plate. The site of this canal is marked by a cross in Text-fig. 1 and, while it is median in position, it is not at the extreme cranial end of the chorda plate which extends more anteriorly to the left of it. I am tempted, however, to regard it as the remains of the chorda canal. Such remains have been described by Eternod (1899) in the embryo Vull, while Rossenbeck (1923) in the earlier Peh-Hochstetter specimen, where there was a chorda canal throughout, found that this passed anteriorly immediately dorsal to the prochordal plate. Bryce (1925) described the chorda plate in the M'Intyre embryo as being continuous cranially with twin 'diverticula', which were dorsal to the prochordal area, but suggested that a cavity in the central mass ventral to these was the blind anterior end of the chorda canal. The channel in my embryo is similar in position to his diverticula, while I suggest that his ventral cavity may well be an instance of the irregular cavitation in the prochordal plate which has been frequently found in it by others. This interpretation would accord with the findings in the Eternod and Rossenbeck embryos.

The cloacal membrane. This begins directly at the caudal end of the primitive streak: there is no interval between the two as in some earlier embryos. Two

sections only show a real fusion of ectoderm with entoderm, which Florian (1932–3) insisted should be regarded as the true definition of the cloacal membrane, but two sections on the cranial side and one on the caudal side of these show outgrowths of ectoderm which in places almost reach the entoderm. In one of the two sections (Pl. 2, fig. 9) a solid outgrowth of the amniotic ectoderm just dorsal to the junction of the amnion and the shield ectoderm is seen to reach and to fuse with the entoderm of the allantois. The other section (Pl. 2, fig. 10) shows columns of cells which, arising from the shield ectoderm close to its junction with the amnion, fuse with the yolk-sac entoderm. These two outgrowths, the one entirely amniotic and the other from the shield ectoderm, are separated from each other by some stalk mesoderm. This is shown diagrammatically in Text-fig. 2. The cloacal membrane is less extensive here than in the earlier Heuser (1932) embryo in which it forms a continuous plate involving both allantoic and yolk-sac entoderm. This is to be expected, since it is generally agreed that the membrane is more extensive in the earlier stages and that its caudal portion gradually disintegrates later. It is, however, unexpectedly more extensive in the Sternberg (1927) embryo with four somites. Here there are three discrete contacts of amniotic ectoderm with the entoderm of the allantois together with one contact of shield ectoderm with the yolk-sac entoderm.

The mesoderm of the disc. There are a few small cavities in the peripheral portions of the streak mesoderm, some of which look more like vascular channels than commencing coelom. These are exceptional, and for the most part the mesoderm appears to be unsplit. In places quite apart from the streak area small bridges of mesoderm unite it to the underlying entoderm, which suggests that the latter may be contributing to its formation.

Yolk sac, body stalk and chorionic cavity. There is nothing in any of these structures that has not been fully described previously. Very marked angiogenesis is to be observed both in the mesoderm covering the collapsed yolk sac and in that of the body stalk. In the chorionic cavity is a very small cyst lined with a cubical epithelium. This is situated close to the chorion and 48 sections (480 μ) away from the cranial border of the yolk sac. Such entodermal cysts have been frequently described (Frassi, Grosser (1912–13) and Heuser) in embryos of about this stage, but, as a rule, they are close to the yolk sac wall.

SUMMARY

The embryonal disc described above measures 1·5 × 1·36 mm. and is reckoned to be 22 days old. In it can be distinguished primitive streak, neurenteric canal, chorda plate and prochordal plate; in the latter area there is apparently a remainder of the chorda canal. The cloacal membrane consists of two distinct portions, the first where amniotic ectoderm fuses with allantoic entoderm, and the second where shield ectoderm extends into yolk sac entoderm. The embryo belongs to the group of which the Frassi embryo is the best known example.

I am indebted to Mr W. Chesterman of this department for much skilled help in my reconstruction and for the preparation of the microphotographs.

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EXPLANATION OF PLATES 1 AND 2

Plate 1

Fig. 1. Photograph of the dorsal aspect of a reconstruction model of the embryonal disc (×75). The amniotic sac has been removed and the allantois dissected out of the body stalk, while the different portions of the axial formations are portrayed on the surface. *P.S.* primitive streak. *N.C.* neurenteric canal. *C.P.* chorda plate. *P.R.* prochordal plate.

Fig. 2. A section (×250) through the primitive streak. It shows the activity of the entoderm which at *X* may be contributing to the mesoderm.

Fig. 3. A section (×250) through the neurenteric canal, *N.C.* *C.P.* indicates the commencement of the chorda plate. *Y.S.* yolk sac.

Fig. 4. A typical section (×250) of the chorda plate, *C.P.*

Fig. 5. A section (×370) showing a mass of cells at *X* contiguous ventrally with the dorsal surface of the chorda plate and dorsally with the ectoderm.

Plate 2

Fig. 6. A typical section through the prochordal plate, *P.R.* (×370) with its characteristic chromatophilic granules.

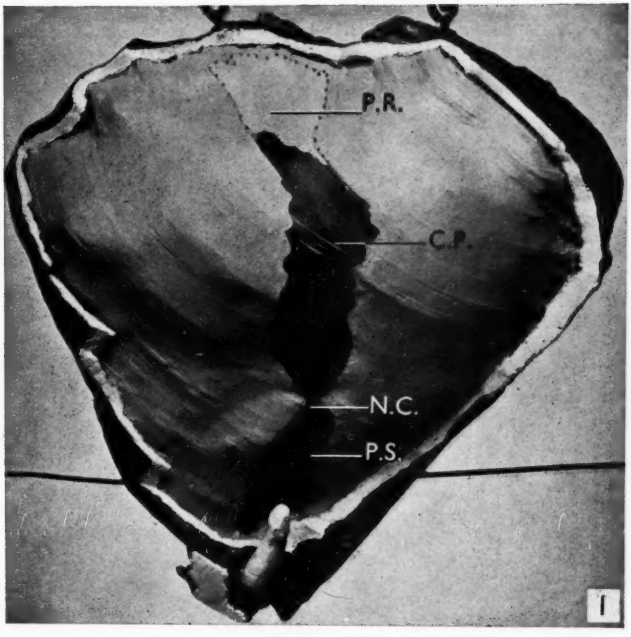
Fig. 7. Section showing the cranial end of the presumed remains of the chorda canal, *C.C.* (×370). Its dorsal wall is seen to become continuous with the entoderm, while ventral to it is the prochordal plate, *P.R.*

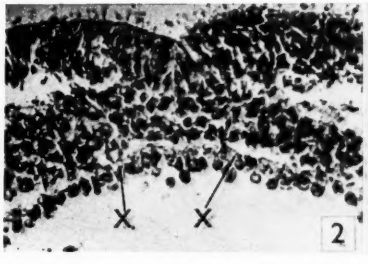
Fig. 8. Section (immediately caudal to that shown in fig. 7) showing the caudal end of the same canal, *C.C.* (×370). Its dorsal wall is seen to be continuous with the chorda plate, *C.P.*

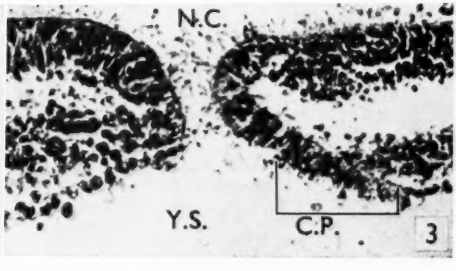
Fig. 9. Section 112 (×250) showing an outgrowth of the amniotic ectoderm, *Am.* fusing with the allantoic entoderm, *AL.*

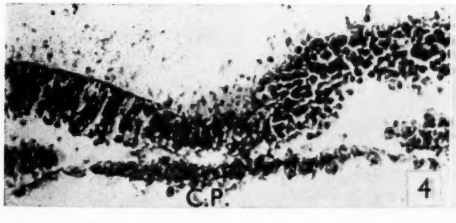
Fig. 10. Section 113 (×250) showing strands from the shield ectoderm reaching and fusing with the entoderm to form the cloacal membrane, *C.M.*

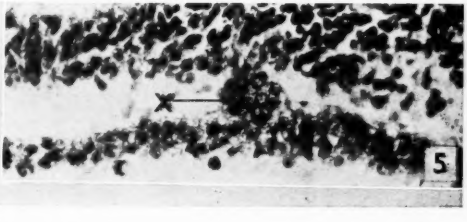
Fig. 11. A section of the embryo in situ (×6).











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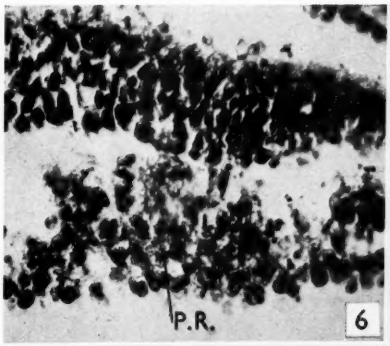

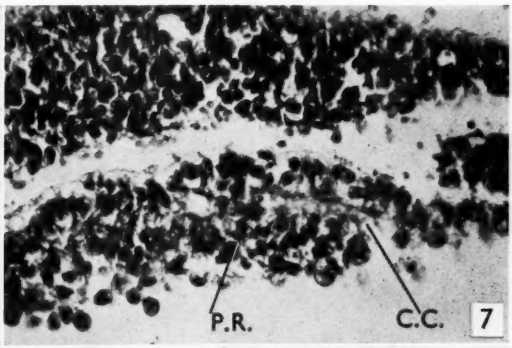

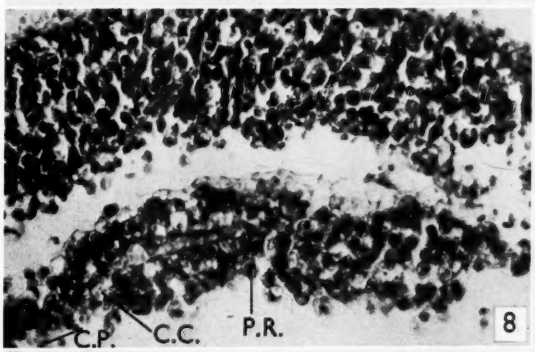

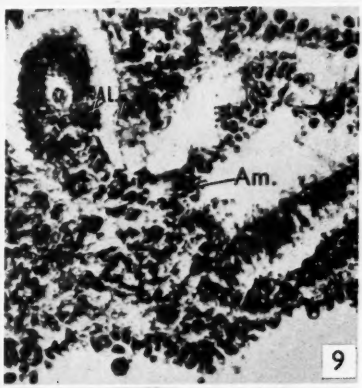

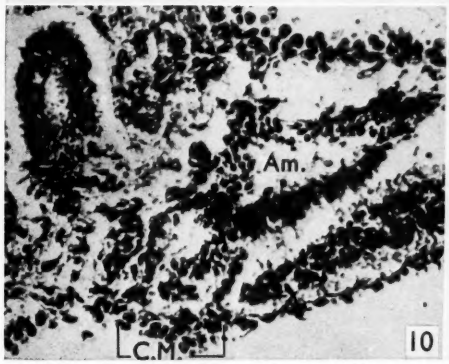

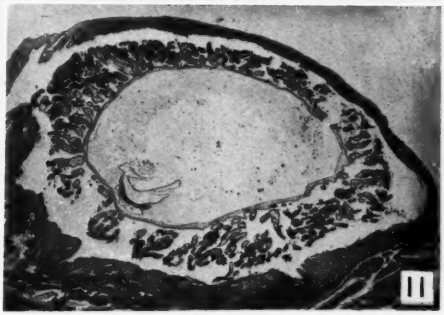

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CONTRIBUTION TO THE STUDY OF THE MORPHOGENESIS OF THE THYROID GLAND

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INTRODUCTION

Many problems concerning the development of the thyroid gland still require study for, in spite of numerous investigators, many of the contributions to its embryology have been based on too small a series of embryos to give significant results. Having at my disposal, through the kindness of Dr V. Patzelt, the large collection of human embryos in the Institute of Histology and Embryology of the University of Vienna[1] I have resurveyed the problem of thyroid development, paying special attention to a peculiar variation in its early anlage and to the question of the developmental history of the thyroglossal duct and its derivatives.

The collection used for this investigation included serial sections of 130 human embryos from 4 to 65 mm. C.R. length and in a good state of preservation. The sections were stained with one or other of the haematoxylin stains or with Mallory's T.C.T. stain. Only those embryos were studied whose condition and plane of section made exact statement possible. Both graphic and plastic reconstructions were made of important stages.

A VARIATION IN THE THYROID ANLAGE

Normally, the thyroid gland appears in embryos of about 17–18 pairs of somites (Davis, 1923; Wen, 1928; Politzer, 1928) as an evagination of a specially differentiated part of the wall of the pharynx into the underlying mesenchyme. It is connected to the pharyngeal cavity by a hollow stalk. In an embryo of 22 pairs of somites, however, Wen described the thyroid primordium as follows: "Just caudal to the first pharyngeal pouch and the bifurcation of the aorta there is a thick walled median invagination of the pharynx, which histologically resembles the thyroid anlage of embryos at this stage of development. It has a core of mesodermal tissue and an endothelial sprout from the ventral aortic trunk extending into it." Wen illustrated this abnormality with a diagram of one section and a mid-sagittal reconstruction.

Our collection of embryos included two embryos showing this abnormality: embryo Bs with 26–27 pairs of somites and embryo F with 28 pairs of somites. Unlike Wen's embryo, of which the head had been removed and in which the thyroid abnormality might be regarded as an artefact, both of our embryos are

[1] This work was completed in July 1938.

sectioned in a complete series and both are excellently fixed and stained. The condition of the tissues permits a reliable diagnosis of the thyroid primordia.

Fig. 1 shows the mid-sagittal reconstruction of a part of the pharynx of embryo F (4 mm., 28 somites). The cavities of the first and second pharyngeal pouches of the right side have been projected on to the mid-sagittal plane in order to show that the thyroid anlage is situated at its normal level. The latter, however, instead of showing the normal evagination, is represented by a small nodule which projects into the pharyngeal cavity. It is formed by an epithelium which comparison shows to be similar in appearance to a normal thyroid primordium (Fig. 2 *a*). There projects into the nodule a mesodermal core containing a small vessel which arises from the aortic sac. The epithelial margins of the nodule are projected like lips towards the underlying connective tissue, and the outer part of these lips merges into the pharyngeal epithelium.

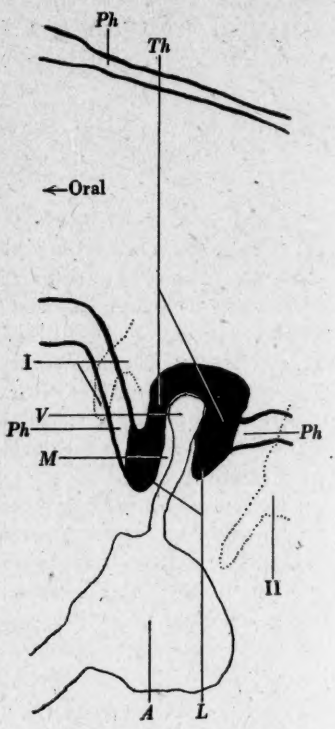

Fig. 1. Mid-sagittal graphic reconstruction of the thyroid anlage of embryo F. The cavities of the first and second pharyngeal pouches of the right side have been projected on to the mid-sagittal plane. *A*, aortic sac; *L*, lipped margin of thyroid anlage; *M*, connective tissue core; *Ph*, pharyngeal epithelium; *Th*, thyroid anlage; *V*, its vessel; I, II, first and second pharyngeal pouches. × 250.

A similar condition is found in the mid-sagittal reconstruction of the pharynx of embryo Bs (Fig. 3). In this embryo the thyroid anlage is smaller than in embryo F and takes the form of a compact nodule with a small mesenchymatous core. The nodule lies partly on the free surface of the pharyngeal epithelium which separates it from the underlying mesenchyme and its conical tip is projected freely into the pharyngeal cavity. The vessel contained in the core of mesenchyme is smaller in this embryo and, as it could not be followed to its source, it is not represented in the reconstruction. The transition from thyroid to pharyngeal epithelium on the lip-like margins of the nodule is similar to that seen in embryo F.

Although the sagittal reconstructions are different, transverse sections through the thyroid primordia of these two embryos are very similar (Fig. 2 *a*, *b*). They differ only in the size of the mesodermal core and in the cephalad inclination of the nodule in embryo Bs.

The vessel which supplies the nodule is noteworthy. Normally the de-

veloping thyroid gland remains for a long time without a special vessel and presumably is nourished through the wall of the neighbouring aorta (Hammar & Hellman, 1920). Figs. 5 and 6 show the relative positions of the normal thyroid primordium and the aortic sac. In some sections of normal embryos,

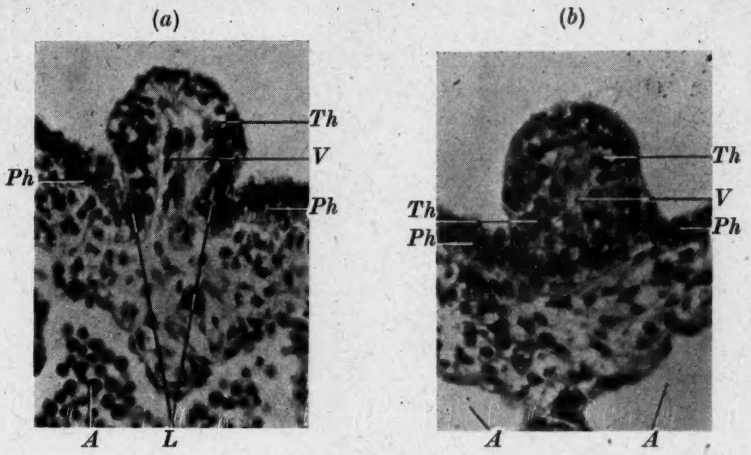

Fig. 2. Transverse section through the thyroid anlage of embryo F (*a*) and of embryo Bs (*b*). *A*, aortic sac; *L*, lipped margin of thyroid anlage; *Ph*, pharyngeal epithelium; *Th*, thyroid epithelium; *V*, vessel to thyroid. × 290.

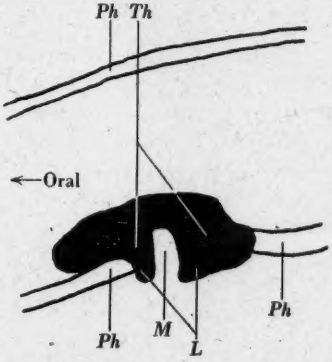

Fig. 3. Mid-sagittal reconstruction of the thyroid anlage of embryo Bs. × 285.

indeed, the thyroid primordium is separated from the blood stream by the vascular endothelium alone. Rabl (1923) assumed that there was a chemotropic effect of the thyroid primordium upon the blood vessels, and this assumption may be applicable in an explanation of the origin of the special vessel found in the abnormal primordia.

The difference between the condition shown by these two embryos and the

normal tuberculum thyroideum must be emphasized. Kallius (1908) described the median thyroid anlage of the rabbit as an epithelial thickening which extends over all of the mesobranchial region. It may become so thick that it forms a hump which projects into the pharyngeal cavity. But where it does so the epithelium forms a cone in the opposite direction reaching the aortic sac. This thickening, called by Kallius the tuberculum thyroideum, is a transient formation which he regarded as serving to "collect" material for the thyroid anlage. It disappears in subsequent development so completely that a small depression appears in its place. It can be seen from Kallius's description and figures that it is a formation quite different from the above-described abnormal primordia. In Wen's embryo, and in mine, there is a distinct invagination of the whole thyroid mass, at the normal level of the anlage, into the cavity of the pharynx. But the tuberculum thyroideum of Kallius is an elevation of the pharyngeal epithelium which extends from the first pair of branchial arches to the level of the third pair of pharyngeal pouches. Moreover, in these human embryos, there is no epithelial cone reaching towards the aortic sac. This cone, which is clearly seen in the rabbit embryo, is found in all mammals at that point where the thyroid gland is formed and represents the chief part of its primordium. Finally, there is nothing in the rabbit embryo corresponding to the mesodermal core and the early vascularization by a special vessel. I conclude, therefore, that the tuberculum thyroideum in the rabbit must not be identified with the rarely occurring invagination of the thyroid primordium into the pharyngeal cavity.

The thyroid primordium described here appears to be a transient formation. It has been found three times among the relatively few described embryos of this stage (22–28 pairs of somites), while no similar formation is known in older embryos. Hence it is concluded that soon after the formation of the nodule a readjustment of the abnormal development is instituted so that the nodule sinks into the underlying mesenchyme and a normal thyroid gland develops. It might possibly be supposed that the anomaly known as struma interlaryngotrachealis could be explained by assuming its origin from the abnormal thyroid primordium described in this section. But struma interlaryngotrachealis is extremely rare, and its connexion with the main thyroid gland and secondary penetration through the wall of the trachea has been demonstrated by Hückel (1932), and further, in my embryos the thyroid nodule is found at the level of the normal thyroid formation and well anterior to the respiratory tract.

THE THYROGLOSSAL DUCT AND ITS DERIVATIVES

In his original description (1885) of the development of the thyroid gland His assumed that the thyroglossal duct passes behind the hyoid bone primordium. He based this opinion on the observation that the pyramidal process of the adult gland is often fixed to the dorsal surface of the hyoid bone. Streckeisen (1886) stated that the thyroglossal duct in development is always

ventral to the hyoid, and he considered that the frequent attachment of the pyramidal process to its deep surface is secondary. Further, he showed that in many cases thyroid glandular material could be found on the ventral surface and above the hyoid bone (pre- and suprahyoid glands). These accessory glands were shown to be frequently connected to the upper end of the pyramidal process by vascular connective tissue bands, by cords of glandular tissue, or by cystic formations which Streckeisen showed to be derived from the thyroglossal duct. He also found in a number of cases a connexion between the suprahyoid gland and the ductus excretorius linguae (Bochdalek, 1866). The suprahyoid gland, or part of it, was found occasionally to be incorporated in the hyoid bone, and transitional forms, from reduplication of the periosteum and inclusion of the gland within its layers to complete incorporation of the glandular material in the bone, were observed. He showed that this incorporation does not occur earlier than the stage of ossification of the body of the hyoid.

In 1891 His recanted his former opinion and adopted Streckeisen's, as he found, in a 16 mm. human embryo, that the situation of the fragments of the duct was such as to make a ventral course of the thyroglossal duct, relative to the hyoid bone, more probable. He wrote: "One gets the impression that the growing hyoid bone had pushed the series of epithelial corpuscles ventrally. I cannot decide whether this impression corresponds to the real process, but I wish to stress one relation—the straight line from the lower end of the cord attached to the tongue to the highest of the epithelial corpuscles beyond the hyoid bone passes directly through the anlage of the cartilage." His described the thyroglossal duct in its upper part, neighbouring the foramen caecum, as a thick stump to which a much thinner thread is attached, the latter being enclosed by a sheath of connective tissue cells.

The opinion of both of these workers soon received opposition. Kanthack (1891), basing his studies on adult material, did not recognize any genetic connexion between the foramen caecum and lingual duct on the one hand and the thyroid gland and its pyramidal process on the other. This opinion was criticized by Marshall (1892) who, in a case of median fistula colli, observed a duct originating in the fistula and connected with the apex of the pyramidal process. From the junction a cord of connective tissue, difficult to distinguish from its surroundings, ran behind the hyoid bone towards the root of the tongue. The upper part of this cord contained a lumen which opened into the oral cavity. On the basis of this finding Marshall repudiated Kanthack's opinion, and, in opposition to His and Streckeisen, he alleged that the thyroglossal duct was situated behind the hyoid bone. However, the basis for this conclusion was only a cord of connective tissue which the author himself admitted was not easily to be defined from its surroundings. Marshall's case has been frequently, but incorrectly, quoted as an example of complete retrohyoid thyroglossal duct.

König (1896) described some cases of median fistula colli. He showed that the thyroglossal duct may be situated in front of the hyoid bone or within two

layers of periosteum, or may even be enclosed in the bone. His description and figures correspond with those of Streckeisen on the incorporation of the prehyoid gland in the hyoid bone, but despite this his cases have been interpreted as showing that the thyroglossal duct was originally within the area where, later, the hyoid bone is formed, that is, that the bone develops around the duct.

In 1896 Schmidt gave a detailed description of the derivatives of the upper part of the thyroglossal duct (the lingual duct or ductus excretorius linguae). In addition he described a thyroid gland with two pyramidal processes, one arising from each lateral lobe. The latter were connected by connective tissue only, a glandular isthmus being absent. The apex of the right pyramidal process terminated abruptly, but that of the left side was continued into a cyst which lay in front of the hyoid bone and reached the lower surface of the genioglossus muscle. The wall of this cyst contained thyroid tissue in its ventral part.

Particular attention attaches to the case published by Matti (1911). Here the thyroglossal duct, which ran behind the thyroid hyoid bone, was patent from the foramen caecum to the pyramidal process and, just above its junction with the latter, opened into the fistula. Matti, referring to His's work, and concluding from his own case, stated that the position of the thyroglossal duct may vary from individual to individual, being situated either in front of or behind the hyoid bone. He suggested that the former relation was the commoner.

Norris (1918) described the early morphogenesis of the thyroid gland. He found three different forms of the thyroid primordium from which he derived different forms of thyroglossal duct. His important investigations will be discussed more fully later in this paper.

Patzelt (1923) described the remains of the thyroglossal duct of some embryos and of one adult woman. He concluded, from the position of the rest of the duct, that it was originally situated in a prehyoid position.

H. Rabl's (1923) ideas on the origin and development of the thyroglossal duct were based on his work on the guinea-pig embryos. He stated that the thyroid gland is not the product of the thyroglossal duct as, for example, a salivary gland is the product of its excretory canal. He wrote: "Obviously the thyroid anlage, when growing caudally, drags behind itself the epithelium of the oral cavity, thus forming the duct. But it is not to be believed that the epithelium of the duct is indifferent, as far as it is adjacent to the specific thyrogenic zone....The tissue which forms the thyroglossal duct is thus a transition between thyrogenic epithelium and the completely indifferent epithelium of the oral cavity."

Wegelin (1926) acknowledged the point of view of Streckeisen and of His, but as one certain case of retrohyoid thyroglossal duct had been observed by Matti, and as Marshall's case had been interpreted in the same way, he concluded: "If the thyroglossal duct fragments the hyoid bone pushes the parts ventrally. But if, when the hyoid bone is formed, the duct is still unbroken, the latter is situated so far dorsally that the bone develops ventral to it."

Fischel (1929) expressed the prevailing view on the development of the thyroglossal duct. He wrote: "The separation of the thyroid gland from the pharynx does not usually happen in the earliest stages, in embryos of 3–7 mm. length the thyroid anlage is still connected to it.... In most cases the thyroglossal duct undergoes involution by losing its lumen in 5 mm. embryos, so that it becomes a solid epithelial cord. In embryos of 7 mm. length this cord is fragmented into several corpuscles of which, in 16 mm. embryos, only rests can be found. These also, for the most part, vanish but it may happen that parts of the duct are preserved."

Schweitzer (1929) described a complete median fistula colli in which he could determine the position of the duct by means of X-ray pictures. Hückel

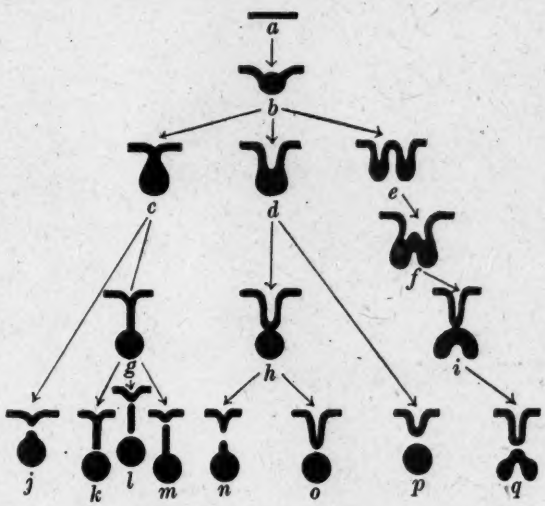

Fig. 4. Scheme of the development of the thyroid gland and thyroglossal duct (after Norris).

(1932) has cited this case as one of a complete thyroglossal duct in a prehyoid position, but Schweitzer's description, which is not accompanied by any figures, is not clear about the relation between the duct and the hyoid bone. Moreover, the case had been complicated by abscesses and, consequently, cannot be regarded as proof of the original relationship.

Kampmeier (1937), in a study on the supernumerary thyroid glands in various mammals, stated that thyroid nodes cephalad to the main gland are characteristic of human embryos. He emphasized that all stages of development may be observed from the fragmentation of the thyroglossal duct into nodes to the differentiation of these nodes into typical thyroid tissue.

In his work on the development of the thyroid gland Norris described three forms of thyroid primordium, one of them being solid (Fig. 4). My material

did not confirm this statement for the primordium was always, at least partly, hollow.

In the embryo B (18 pairs of somites) the thyroid anlage is represented by a small pit with thick walls (Fig. 5). All the embryos (eleven in number) in my series between this one and a 6·5 mm. embryo have a hollow duct. Fig. 6 illustrates such an embryo (Fu, 28–29 somites) which shows an extension of the pharyngeal cavity into the thyroid primordium. This stage corresponds to the stage *d* in Norris's scheme (Fig. 4). The distal part of the primordium is solid and contains cells more compactly arranged than in the proximal part. The latter can be considered as the anlage of the thyroglossal duct, while the distal part is probably that of the gland itself. The epithelium of both the

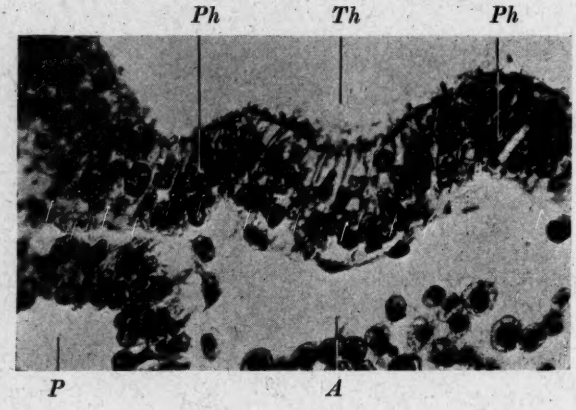

Fig. 5.

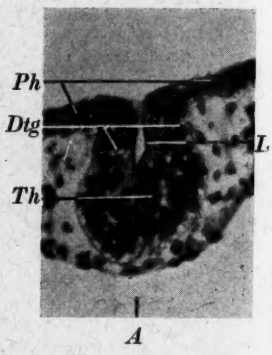

Fig. 6.

Fig. 5. Section through the thyroid anlage of embryo B. The wall of the pit represents the anlage of both the thyroid gland and the thyroglossal duct.

Fig. 6. Section through the thyroid anlage of embryo Fu with 28 pairs of somites. *A*, aortic sac; *Dtg*, thyroglossal duct; *L*, its lumen; *Ph*, pharyngeal epithelium; *Th*. thyroid gland. × 290.

glandular and duct portions of the primordium is always different from that lining the pharynx. Comparison between Figs. 5 and 6 shows that the duct and gland are formed simultaneously as a small pit with a differentiated epithelial lining. They can be distinguished from each other only at that stage when, by deepening and narrowing of the cavity, the pit becomes a narrow passage between the pharynx and the gland primordium. At the same time the histological differentiation commences. Later the thyroglossal duct becomes longer and thinner and its lumen narrower. The lumen is limited to the duct and never enters the gland primordium. At this stage the appearance is similar to that of stage *g* in Norris's scheme. The embryo Bb of 6·5 mm. length and all the older embryos in my material have a solid duct.

Before fragmentation the thyroglossal duct consists of a specific epithelium which, as development proceeds, becomes longer and, at the same time, much thinner, especially in its intermediate part, and its lumen is obliterated. Finally, in transverse sections, it is represented by a few cells only. These cells are large and vesicular with a palely staining protoplasm and a central nucleus. The duct can be distinguished only with difficulty from the surrounding connective tissue. The glandular tissue, on the contrary, is formed by small cells with darkly staining cytoplasm and can readily be recognized even in small cords. At those points where the thyroglossal duct is about to fragment the basement membrane is not recognizable and the cells show degenerative changes.

Table 1

Length of embryo in mm.*	No. of embryos with complete duct	No. of embryos showing forms			No. of embryos with duct absent
		l	*m*	*n*	
7	3	/	1	2	/
7·5	3	/	1	/	/
8	2	/	/	1	/
8·5	1	/	2	/	/
9	3	/	2	2	/
9·5	1	/	2	/	/
10	2	1	1	4	/
10·5	/	/	/	1	/
11	/	/	4	4	2
12	/	1	1	9	1
13	1	/	2	4	1
14	/	/	1	3	/
15	/	/	1	/	/
16	1	/	/	3	/
17	/	/	2	2	/
18	/	/	1	1	/
19	/	/	/	2	1
20	/	/	1	4	/
21	/	/	1	1	/
22	/	/	2	1	/
	Total 17	2	25	44	5

* If the length of an embryo was between two successive numbers it was ascribed to the smaller length.

Norris distinguished eight forms in which the thyroid gland may be separated from the pharynx (Fig. 4 *j–q*). Only three of these are shown in my material, *l*, *m* and *n*. Table 1 shows the relative frequencies of each of these: *n* is more frequent than *m*, while the other forms are very rare. The table also shows that only two unfragmented thyroglossal ducts were found in embryos of more than 10 mm. length.

In conclusion it can be stated that the thyroglossal duct is formed simultaneously with the gland. During the migration of the gland it becomes longer and thinner and the lumen disappears. Finally the duct fragments, in most cases at about its mid-point. This process is usually completed by the 10 mm. stage.

DEVELOPMENT OF THE PYRAMIDAL PROCESS

Embryos of 7 mm. length do not yet possess a glandular pyramidal process, the thyroglossal duct passing directly into the thyroid gland. It is only in embryos of 7–10 mm. that the lower part of the duct differentiates into glandular tissue. The pyramidal process thus produced is at first short with its cephalic end continuous with the cord of duct tissue. The latter becomes progressively shorter as the glandular part grows. Apparently the duct tissue differentiates into glandular tissue but the delimitation of the two portions from each other is more or less clear. The total length of pyramidal process and cord of duct tissue fluctuates according to the position at which fragmentation of the thyroglossal duct first occurs. The pyramidal process usually has a broad connexion with the thyroid gland, but the connexion may be represented by a few cords of cells only. Occasionally it may be entirely separated from the gland.

There is considerable variation in the cephalic end of the pyramidal process in embryos of more than 10 mm. length. It may pass into a cord of duct tissue, the upper portion of which may be fragmented, or it may end in a swelling. In older embryos this swelling may no longer be in connexion with the pyramidal process, in which case it is represented by an accessory glandular node in front of the hyoid bone (prehyoid gland).

The pyramidal process is at first median but after the 10 mm. stage it comes to lie laterally. The thyroid gland itself is uninfluenced by this displacement of its pyramidal process, the isthmus remaining in the median plane. There are large individual variations, but in older embryos the pyramidal process usually lies far laterally. There is about an equal probability of the displacement being to the right or to the left. In only one older embryo (Ck, 21 mm. long) was the pyramidal process found to lie exactly in the median plane.

The direction of the displaced pyramidal process varies; it may be directed cephalad, parallel to the median plane, or it may pass towards the median plane which it may reach. In the latter case it may continue in a cephalad direction after having reached the median plane. It has never been seen to pass to the other side.

The separated pieces of the thyroglossal duct cephalad to the pyramidal process may show a lateral displacement to the same side as the pyramidal process or they may lie in the median plane.

One embryo in our collection showed a doubling of the pyramidal process (Bw, 11·5 mm. long). In this embryo both the foramen caecum and the lingual duct are absent. The lobes of the thyroid gland are in a normal position but are not connected by an isthmus. A pyramidal process arises from the medial surface of each lobe and these processes are directed towards the middle line where they meet. Together, therefore, the pyramidal processes form an arch, the upper part of which is thyroglossal duct tissue while the parts adjoining the thyroid lobes are glandular.

In conclusion we may say that the pyramidal process develops out of the lower part of the thyroglossal duct by differentiation of the duct tissue into gland tissue. It is only after this differentiation has commenced that the lateral displacement of the pyramidal process begins. The accessory thyroid glands, as far as they have taken origin from the pyramidal process, may show an equivalent displacement.

DEVELOPMENT OF THE LINGUAL DUCT

The term lingual duct can only be used after the thyroglossal duct has divided and only if the fragmentation has occurred beneath the surface of the tongue. Table 1, column *n* shows that the lingual duct is found, after division of the thyroglossal duct, in more than half of the specimens.

At first the lingual duct has exactly the same appearance as the thyroglossal duct, being formed by the specific epithelium described above and containing no lumen. It is situated in the median plane, has a variable length but a fairly uniform width. Fragments of tissue may be separated from it, as from the pyramidal process, and these may remain as cords of duct tissue, as accessory thyroid glands or as cysts (see later).

The uniform width of the lingual duct does not persist. His (1891) described the proximal part of the lingual duct of an embryo of 16 mm. length as a thick stump to which a much thinner thread is attached. To this observation of His I should like to add that this broadening of the proximal part of the lingual duct begins in embryos of 10–11 mm. length. Moreover, while the lingual duct is at first formed by the specific epithelium of the duct, in embryos of approximately 13 mm. length the epithelium of the thick proximal part begins to resemble more and more the neighbouring epithelium of the tongue. In embryos of about 20 mm. length a cavity is formed in this thick stump. This cavity represents the definitive foramen caecum and is to be distinguished from the foramen caecum of young embryos which is a transient formation only.

The distal part of the duct does not undergo corresponding changes. It does show some thickening but the thickening is not accompanied by a change in the character of the epithelium. At about the 30 mm. stage, however, small branches of the lingual duct arise from this non-stratified portion of its course. These are the so-called tubes of Bochdalek. They have been found in all of my embryos more than 30 mm. in length which possess a lingual duct. While the lingual duct itself is situated in the middle line, the branches, which grow ventrally, may curve to the right or to the left. The number of these ducts varies and their length increases with age. Histologically they resemble the sprouts of salivary glands. The difference between them and the lingual duct is shown in Fig. 7. Similar branches may arise from the fragments of the thyroglossal duct which have separated from the caudal end of the lingual duct.

In conclusion it may be stated that the lingual duct is the upper part of

the thyroglossal duct. Its proximal part thickens, changes into an epithelium resembling that of the tongue and develops a cavity. From its distal portion which retains its specific histological character, branches grow out which come to resemble salivary glandular material. Examination of older embryos shows that the transformation of the epithelium gradually proceeds down its distal portion. At the same time the cavity of the foramen caecum lengthens, forming a hollow duct (secondary cavity). Thus the salivary-gland-like ducts come to open into a hollow duct lined by stratified epithelium.

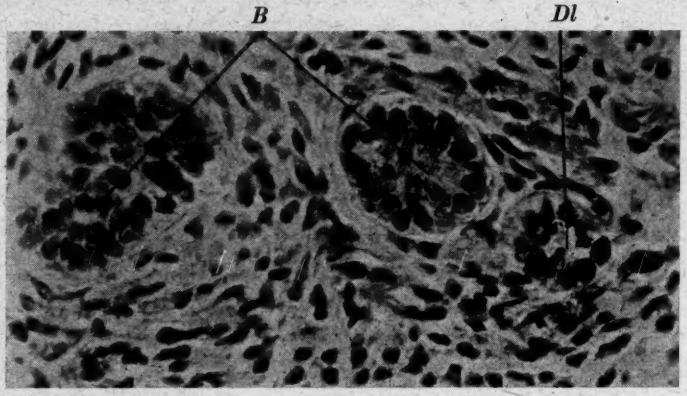

Fig. 7. Section through the lingual duct of embryo Ez, 31 mm. long. *B*, branches of the lingual duct; *Dl*, lingual duct. ×200.

THE POSITION OF THE THYROGLOSSAL DUCT RELATIVE TO THE HYOID BONE

According to my observations the hyoid bone develops dorsal to the thyroglossal duct. The bone first appears, in embryos of 12–14 mm. length, as a condensation of connective tissue. By this time the thyroglossal duct has already commenced to fragment, but it can be clearly seen that the hyoid bone is limited ventrally by the fragments of the duct. This may be observed in sections in every plane, but especially well in sagittal ones. By the ventral and caudal enlargement of the hyoid bone the cephalic fragments of the duct are displaced ventrally, whilst the caudal ones come to lie behind the hyoid bone. This assumption of His was confirmed by the investigation of a large series of embryos of different stages. Further, the fragments of the thyroglossal duct are often connected with each other ventral to the hyoid bone by cords of connective tissue which represent the sheath formerly enclosing the thyroglossal duct. Finally, one of the embryos (Bp, 16 mm. long, Fig. 8) retained a complete thyroglossal duct although the hyoid primordium had already appeared. The graphic reconstruction shows clearly that the duct is displaced ventrally by the hyoid. At the point of greatest displacement the

duct is thinnest. In this embryo the hyoid anlage consists of two parts; one is precartilage, the other is still dense connective tissue. Ventrally it can be sharply distinguished from the connective tissue but dorsally it passes gradually into the denser mesenchyme. The examination of the human embryos at my disposal showed that in all of them the position of the fragmented thyroglossal duct corresponds to the relations shown in Fig. 8.

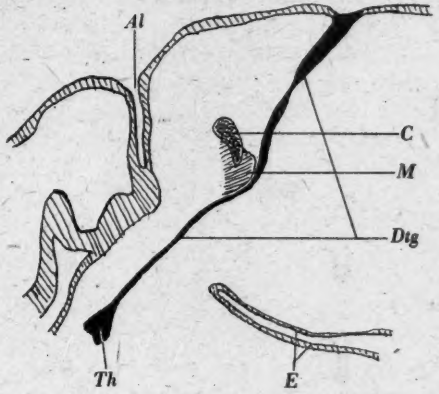

Fig. 8. Reconstructed thyroglossal duct of embryo Bp, 16 mm. long. *Al*, aditus ad laryngem; *C*, precartilage part of hyoid anlage; *Dtg*, thyroglossal duct; *E*, epidermis; *M*, connective tissue part of hyoid anlage; *Th*, thyroid gland.

DISCUSSION

The development of the thyroid gland begins in embryos with 17–18 pairs of somites by the formation of a small pit (Figs. 5, 6) in that place where the first pair of pharyngeal pouches meet (Norris represented this stage differently, Fig. 4 *b*, but I think that only cases of double thyroid anlage, in their early stage, correspond to his *b*, see Fig. 9 *b* 2). This pit is lined by a specific epithelium which is the anlage of both thyroid gland and thyroglossal duct. By the lengthening and narrowing of this pit the form represented in Fig. 6 (see also Fig. 9 *d*) is produced. Although there is no sharp limit between them the gland anlage and the duct anlage can be easily distinguished from each other.

It appears that the lengthening of the duct is not caused by active growth but by passive extension. This is suggested by the narrowing of the lengthening duct, which loses its lumen in the process, and by the absence of mitoses in its cells at this stage. At some points the cells die and as a result the thyroglossal duct becomes divided into fragments.

Norris stated that the solid thyroglossal duct (Fig. 4 *g*) develops from the solid anlage (*c*) and that the hollow duct (*h*) arises from the hollow anlage (*d*). My findings are contrary to this opinion for all my embryos of less than 6·5 mm. length had a hollow duct and all the older embryos have solid ducts. Hence I conclude that the solid duct develops from the hollow duct by extension.

I agree with Norris in deriving forms *l* and *m* from *g*, but I do not believe that form *n* develops directly from form *h*. I never found a trace of the division of a hollow duct. Further, I observed in many embryos with a solid duct that it was thinnest in its intermediate portion and that there its cells appeared degenerated. It seems, therefore, certain that form *n*, as well as forms *l* and *m*, develop from *g*. None of the other forms described by Norris (*j*, *k*, *o*, *p*, *q*) of separation of the thyroid gland from the pharynx were observed in my material. By disregarding them, the scheme of the development of the thyroid gland becomes much simpler according to my findings (Fig. 9) than is the one given by Norris (Fig. 4).

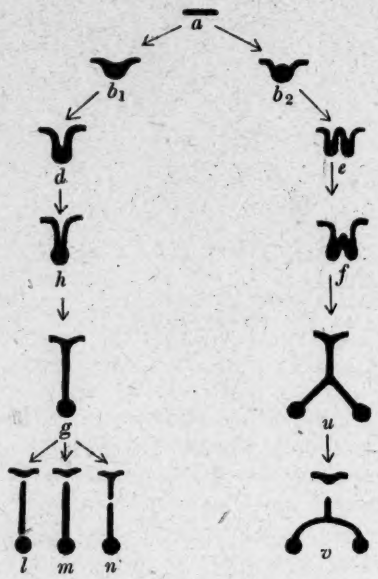

Fig. 9. Scheme of the development of the thyroid gland and of the various forms of thyroglossal duct rests.

Tourneux & Verdun (1897), having only a few specimens at their disposal, supposed the pyramidal process to appear first in embryos of 26 mm. length. According to them it arises from one of the lateral lobes. This opinion has not been accepted and I could find no evidence for it. Nevertheless, their opinion is understandable for the thyroglossal duct originally joins the gland in the middle line, whilst later the pyramidal process is attached to one of the lateral lobes. But the examination of a large number of embryos of different stages shows clearly the transformation of the duct epithelium into glandular tissue and all stages in the transition from a median to a lateral pyramidal process.

Finally, the embryo Bm, described above, may be considered as a reliable proof against Tourneux & Verdun's interpretation. In this embryo a thyroglossal duct is preserved throughout its length and shows in its distal portion a glandular transformation and a lateral displacement similar to that of all pyramidal processes at this stage of development.

The rare anomaly of a double pyramidal process is generally explained by assuming a high bifurcation of the thyroid anlage, that is, it is supposed that the growing thyroglossal duct splits at its apex and that each branch gives origin to a lobe of the gland. This view is not supported by the facts that the gland and the duct originate simultaneously, that the gland is not the product of the duct, and that the duct becomes longer by passive extension only and not by active growth. Fig. 25 of Weller (1933) shows a doubling of the thyroid anlage and Norris presented a similar, and an older, stage in his scheme (Fig. 4 *e, f*). In my opinion such a doubling of the anlage of the thyroid means also a doubling of the thyroglossal duct, for each of the evaginations is gland anlage in its distal portion only, being duct primordium in its proximal part. I consider that from Norris's stage *f* a thyroid gland with a double pyramidal process may be easily derived whereas Norris and Weller derive only an early bilobed thyroid gland from it. On my interpretation, the common proximal part of the duct as well as its double distal portion is passively extended and the appearance of bifurcation of the pyramidal process, when it differentiates, results (Fig. 9; see also Weller, Fig. 5). Later the two "branches" of such a bifurcate thyroglossal duct may (case of Schmidt) or may not (embryo Bw, Fig. 9 *v*) be separated from one another. The examination of the embryo Bw shows further that cords of thyroid cells have grown medially and laterally from each gland primordium. I think it is probable that the medial lobes of each of these glands would later have united to form a secondary isthmus. Thus an isthmus may be found in some cases of double pyramidal process, whilst in others it can be missing (Hückel's case).

The fact that the hyoid gland of the guinea-pig, originating from the thyroglossal duct, is slower in its development than the main thyroid gland, and differs from it in structure, led H. Rabl to conclude that the thyroglossal duct develops from a tissue not equivalent to thyroid tissue. He supposed the thyroglossal duct to be oral cavity epithelium subjected to traction by the migrating thyroid gland, and he considered this epithelium to represent a transition between the thyrogenic and the "indifferent" oral epithelia. On the basis of this observation Rabl supposed that in man the "indifferent" epithelium of the oral cavity also participates in the formation of the upper part of the thyroglossal duct. He considered that the stratified epithelium and the mucous glands derived from the lingual duct supported this view. My observations, however, show that, in the human embryo, the epithelium of the thyroglossal duct in its earlier stages resembles thyrogenic epithelium more than the oral epithelium. The undivided thyroglossal duct is lined by this specific epithelium up to its junction with the tongue. Only after frag-

mentation of the duct does its proximal portion show a transformation of its epithelium to one similar to the tongue epithelium. It cannot be concluded that the latter is dragged or grows actively into the connective tissue of the tongue, for, after separation of the thyroid gland, there is no fixed point to enable the lingual duct to drag the epithelium and there is no sign of active growth. I conclude, therefore, that the epithelium of the thyroglossal duct is a specific one, but from the point of view of its potentialities, it is intermediate between the thyrogenic and the oral cavity epithelia. Its lower end changes into thyroid tissue and its upper end differentiates to resemble the neighbouring epithelium of the tongue.

The mucous glands of the lingual duct constitute no proof that the epithelium of the duct is common oral cavity epithelium, for the glandular sprouts arise directly from the specific duct epithelium (Fig. 7) and not from stratified epithelium.

In my material no cysts of the lingual duct were found, but one embryo (Ge, 43 mm. in length) showed a separated fragment distal to the lingual duct itself, both being completely lined by stratified epithelium. Branches have grown out from the fragment which is already hollow. It seems probable that after the beginning of secretion by the differentiated mucous glands such a formation becomes a cyst.

The position of the thyroglossal duct relative to the hyoid bone seems to be quite clearly defined by my material. The duct itself is situated in front of the region where later the hyoid bone is formed. Its fragments lie in front of the hyoid bone and may be included in the bone, in later stages, either as long cords or accessory glands (Streckeisen, König).

Difficulties in the way of accepting this interpretation are provided only by the case described by Matti, and by Wegelin's supposition, based on this case, that the fragments of the divided thyroglossal duct lie in front of the hyoid bone, but that the duct if unfragmented at the period of formation of the hyoid, lies so far dorsally that the bone develops ventral to it. This statement, like others in Wegelin's book, shows that he dealt with embryological problems in a purely theoretical manner. Examination of embryos at different stages shows that the thyroglossal duct before fragmentation lies in the same plane as its fragments afterwards. Only after the formation of the hyoid bone has begun are the fragments pushed ventrally by it. Nevertheless Matti's case exists and cannot be denied. But even if, considering this case an anomaly, we set aside the findings of normal embryology and do not take into account the embryo Bp, which has an undivided thyroglossal duct running in front of the hyoid bone, Wegelin's conclusions must still be opposed on the basis of Matti's own findings. The duct opened into a fistula. This alone shows that the duct was in an abnormal position, being too ventral in its lower portion and, presumably, too dorsal in its upper. The cause of this is unknown but it must be emphasized that in this case there was an anomaly of position as well as the anomaly of persistence of the duct. And a combined anomaly cannot be

an adequate basis for a general conclusion which would assume the second anomaly to be the rule every time the first anomaly occurs.

SUMMARY

1. A variation in the thyroid anlage is described in two human embryos. The variation consisted of an invagination of the thyroid anlage into the pharyngeal cavity and it is suggested that, if development had proceeded, the abnormality would have righted itself and developed into a normal thyroid gland.

2. The developmental history of the thyroglossal duct, the lingual duct and the pyramidal process of the thyroid gland, is described for a large series of human embryos from the 4 mm. to the 65 mm. stage.

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CYTOLOGICAL CHANGES IN SENILITY IN THE TRIGEMINAL GANGLION, SPINAL CORD AND BRAIN OF THE MOUSE

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INTRODUCTION

The nervous system is of particular interest in the study of cytological changes in senility because the units of this system, the neurones, maintain their identity from birth to old age. They can thus be expected to reflect the general changes in the organism. In earlier studies (Andrew, 1936, 1937, 1939*a*) some of the age changes in the brain of the mouse were demonstrated. Our previous work on this animal has been confined to the cerebellar and cerebral cortex. The object of the present study is to determine whether changes similar to those found in these regions, or changes of a different nature, could be demonstrated in other regions of the nervous system.

The trigeminal or semilunar ganglion was chosen as an object of study because it was felt that this may readily typify the changes which might be expected in other ganglia and because of its relatively large size, which makes it convenient for dissection and removal even in a small laboratory animal such as the mouse. The ventral horn, with its large motor cells, with their well-known structure, connexions, and function, seemed the most logical region of the spinal cord upon which to concentrate.

Literature is not lacking on age changes in these two regions; but it must be pointed out that much of it is from a rather early period in histological research and that the changes in one part of the nervous system of an animal have not often been compared with changes in another part. Comparative work with human autopsy material, and with animals whose tissues can be removed almost immediately after death, also seems much to be desired.

The work on the spinal cord dates back to the close of the past century. Nonne (1899) studied the spinal cords of ten senile persons, all but two of whom had only senility with intercurrent pneumonia as the cause of death. In none of these persons were there any special symptoms. He found a definite increase in blood vessels in some parts of the cord and increase in thickness of the walls in others. There was an increase in glial cells in the white matter, and the nerve fibres seemed to be crowded and crushed by these elements. He did not stress the changes in the grey matter, but apparently believed the degenerative changes here to be secondary to those in the white matter.

Sander (1900) studied thirty senile spinal cords from autopsy specimens, ranging in age from 51 to 87 years. He found in every case changes in the grey

matter, especially in the ganglion cells, as well as in the white matter. The most common form of degeneration was that which he describes as 'pigmentary'. Here, he says, the Nissl bodies dissolve themselves into a yellow-brown pigment, the process generally beginning at the axis cylinder end of the cell. The nucleus becomes dark, and migrates to an excentric position, and the nucleolus grows irregular and somewhat shrunken. Finally, nucleus and nucleolus disappear and there remains only a rounded pigment-containing mass. This itself finally disappears in many cases, leaving only a space in the tissue.

He observed also two other types of degeneration: a 'sclerosis', in which the cell body and nucleus become light-coloured and little-differentiated, and another type in which both nucleus and cell body are very dark, the Nissl bodies forming a scarcely distinguishable clump.

In both cases of persons over 85 years old which he examined, he found scarcely a single ganglion cell in the grey substance which could be described as normal. He does not describe the relationship of the glial cells to the nerve cells in his specimens.

Harms (1924) found very definite changes in the spinal cord as well as in other parts of the nervous system of senile dogs. Chief among the changes in the cord were the heaping up of pigment, loss of regularity of outline of the cell body, and loss of distinctness of the boundary between nucleus and cytoplasm. He also speaks of 'numerous phagocytes' among the ganglion cells.

Fluegel (1927) reported on the cords of six persons over 80 years of age who died from cerebral haemorrhage or from pneumonia. He found a definite increase in glial nuclei in the grey matter, but found it rare to see these in the form of 'rod-cells'. Of the nerve cells, he says (p. 620): 'Dans la plupart les grains tigroides sont délabrés et souvent agglomérés en masses plus ou moins volumineuses. Les noyaux sont souvent fort colorés et ne presentent pas des détails chromatiques nets.' He found an accumulation of pigment in all of the nerve cells. The changes were most pronounced in the motor cells. Concerning phagocytic activity in the cord, he says (p. 622): 'L'activité éliminatoire de la neuroglie est restreinte. Une collaboration mésodermique n'est pas visible.'

Stankiewitsch (1934), in a study of age changes in human nerve cells, found pigmentation in the spinal cord and spinal ganglia and vacuolation in the nucleoli in a 70-year-old woman.

A recent article by Stern (1936) states that there are two main types of lesions within the grey matter in old age: namely, a focal degeneration of ganglion cells due to vascular changes, and a pigmentary atrophy of the ventral horn cells of a systemic character. He states also that there is a glial fibre proliferation in certain regions, the ventral horn among them, but that this proliferation bears no relation to the parenchymatous changes and is not of vascular origin. Critchley (in Cowdry, 1939, p. 486) considers the most important aspect of changes in the nerve cells in old age to be the increase in pigmentary elements.

Work on the number of nerve fibres in the ventral roots of rats at various ages may be correlated with the findings on degeneration of the ventral horn

cells. Thus Duncan (1934) concludes in regard to the fibres that there is a slight decrease in number in old age amounting to one-tenth the total number at 800 days.

In regard to the changes in the semilunar ganglion with age, the literature is at present almost lacking. We do, however, have data on changes in some other ganglia (Delorenzi, 1931; Kuntz, 1938), and some recent work is accumulating on age changes in the semilunar ganglion in man (Truex, 1940). The latter has found a very definite vacuo-granular degeneration in these cells, the vacuoles appearing to grow so large in many instances as to occupy practically all of the space previously occupied by the cytoplasm of the cell.

Kuntz (1938) reported on changes in the autonomic ganglia with age and in disease. He includes under age changes (p. 793), 'changes in the chromidal content of ganglion cells associated with normal functional activity, deposition of melanotic pigment in moderate amount in some ganglion cells, and degenerate changes in occasional cells, particularly in advanced age'.

Of the numerous types of degenerative processes which affect the nerve cells of the ganglia, as described in detail in De Castro's summary of the subject (De Castro, 1932), a certain number are strongly suggestive of the pictures seen in cells in other parts of the nervous system in senility, and we had little doubt that some of these changes would be found in old age in the ganglia of the mouse.

MATERIALS AND METHODS

The animals used in this study were eleven pedigreed black mice of the strain C 57 from the Jackson Memorial Laboratory at Bar Harbor, Maine. This is the strain used in our earlier work, in which the brains of some forty to fifty of these C 57 mice have been studied.

The age in days and sex of these animals were as follows: 40-day ♂, 41 ♀, 312 ♂, 362 ♂, 586 ♀, 595 ♀, two of 596 days, ♀'s, 693 ♀, 696 ♀, and 699 ♀. Thus there were two very young animals, two which we might class as 'middle-aged', and seven which were definitely senile.

The animals were killed by rapid etherization. In earlier work decapitation had been used, but this did not seem feasible if a proper dissection and removal of a part of the spinal cord in these small animals was to be made. In the earlier studies (Andrew, 1936) we had found no definite effect on the nerve cells from such rapid etherization.

The brain and the cervical and part of the thoracic cord were exposed as rapidly as possible and removed in one piece, usually within 5 min. after death. The cord was then cut from its junction with the medulla and fixed in 10 % formalin for Nissl staining. The brain was divided in the median sagittal plane. The left half was placed immediately in a formalin-ammonium bromide solution (formalin, 56 c.c.; ammonium bromide, 8 g.; distilled water, to make 400 c.c.) for silver preparations of the glial cells. The right half was fixed in 10 % formalin for Nissl staining.

The semilunar ganglia had been exposed as the brain was lifted up with the spatula, and these were now removed from both the right and left side, and fixed in 10 % formalin for Nissl staining.

The above procedure was followed in all but three of the animals, namely, those of 362, 586, and 696 days respectively. In these animals the spinal cord, semilunar ganglia, and the right half of the brain were fixed in Da Fano's cobalt nitrate and formalin solution for demonstration of cell outlines and Golgi apparatus. The left half of each brain was, as in the other animals, placed in the formalin ammonium bromide solution.

The tissues were carried through the technical processes in such a way that those of some of the younger animals always accompanied those of senile animals, in the same tray with the same fluid. Thus for the Nissl staining the tissues from the 40-day ♂, the 693-day ♀, and 595-day ♀ were taken through in one tray; those from the 41-day ♀, and the two females of 596 days in another; and those from the 312-day ♂ and 699-day ♀ in a third. A similar procedure was followed in the Da Fano and glial cell preparations, so that each senile animal would have a control young animal which had been run through in the same dish of solution.

The Nissl and Da Fano material, after the preliminary steps in these processes, was dehydrated, cleared, infiltrated, embedded in paraffin, sectioned at 8 μ, and mounted on slides. Transverse sections were cut of the cord, and longitudinal sections of the ganglia. The paraffin was then dissolved from the Da Fano sections and the slides mounted in balsam. The Nissl material was stained for 75 min. in a 1·5 % aqueous solution of cresyl violet. Sections from the tissues of the same animals, grouped together in the earlier stages of the technique up to embedding, were carried through the xylol, alcohols, and stain in the same glass slide-tray.

In regard to the preparation of the sections for demonstration of the types of glial cells and their processes, we used a modification of the silver method of Rio-Hortega. Our method was as follows:

(1) Fix in formalin-ammonium bromide solution for 24 hr. to several days.

(2) Cut frozen sections of brain (sagittally, in this case) at 20–25 μ.

(3) Place in 1 % formalin for temporary storage.

(4) Deformalinize 1 min. in dish of distilled water which has about 1 drop of strong ammonia for each 5 c.c.

(5) Wash in distilled water.

(6) Place section in a 5 % sodium carbonate solution for 1 hr.

(7) Wash in distilled water.

(8) Impregnate in silver carbonate solution.

We used a solution like that of Rio-Hortega, but containing 10 g. of silver carbonate per 450 c.c. of distilled water, rather than the 5·4 g. of sodium carbonate used in his 'weak solution' and recommended in Penfield's 'combined method'.

The impregnation was carried out *in the incubator* at a temperature of 38° C. for a period of 12–15 min. Heating during impregnation was a feature of the Spanish methods (Da Fano, 1926).

(9) Rinse in distilled water.

(10) Reduce in 5 % formalin until the sections are a yellowish grey.

(11) Wash in distilled water.

(12) Tone in gold-choride (1 : 500).

(13) Fix in 5 % photographic hypo, 5 min.

(14) Wash in distilled water.

(15) Dehydrate on slide with 95 % alcohol followed by carbol-xylol-creosote mixture (1 : 15).

(16) Mount in balsam.

This method is the result of a number of trials with the mouse brain and gave rather good results with astrocytes and oligodendroglia, although not with absolute constancy.

OBSERVATIONS

We shall consider first the findings on the spinal cord and semilunar ganglia. In the spinal cord of the 40-day ♂ and the 41-day ♀ mouse, the majority of the cells have a considerable amount of Nissl material in large blocks throughout the cytoplasm. While the nuclei in the smaller cells are often basophilic, those of the larger ventral horn cells often show as clear, white vesicles with the deeply staining nucleolus standing conspicuously in the centre. Instances of satellitosis are not numerous and, where they occur, there are usually but one or two glial cells in contact with the body of the nerve cell. We found no pigment in any of the motor cells in these animals. There are no nuclear aberrations, no marked vacuolation or dissolution of the cytoplasm, and no neuronophagia.

In the spinal cord of the 312-day male mouse there are somewhat fewer motor cells with large amounts of Nissl material. Vacuolation of the cytoplasm was visible in a very small percentage of the cells. By careful examination small amounts of yellow-brown pigment could be seen in some cells, but in under 5 % of the total number. The nuclei are usually lightly basophilic. The very clear whiteness of the nuclei in some of the larger cells in the two youngest specimens is not found here.

The Nissl picture seen in the case of the senile animals, the 595-day ♀, the two females of 596 days each, the 693-day ♀, and the 699-day ♀, while not exactly uniform in these different animals, shows in each of them marked and consistent differences when compared with that in the three younger animals. The number of hypochromatic cells in the senile animals is much greater. In the two oldest animals chromatic cells actually are rare, only one cell with a normal amount of Nissl material being present among several dozen of the large motor cells. In all of the animals vacuolation and shrinkage of the cytoplasm are seen in many of the cells. The type of vacuolated, hypochromatic cell is well seen in Text-fig. 2.

The accumulation of yellow-brown pigment in the motor cells is conspicuous in all of the old animals. In the two oldest animals practically every cell has some pigment, and the majority have half or more of the volume of the cytoplasm obscured by it.

The nuclei of all of the cells in these senile animals are basophilic and the nucleoli are pale and often rather poorly differentiated from the ground substance of the nucleus.

Actual degeneration and 'death' of the cells are readily observed. In this regard, it is of great interest to note that the satellite cells here seem to play a

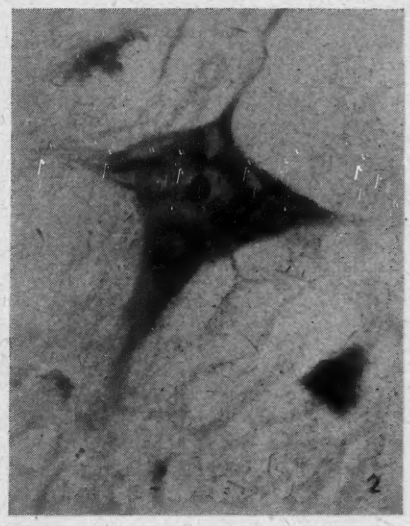

Fig. 1. A motor cell from the ventral horn of a young male mouse, 40 days of age. Nissl stain. The Nissl material is abundant and the nucleolus deeply staining. ×1200.

Fig. 2. A motor cell from the ventral horn of a senile female mouse, 596 days of age. Nissl stain. The cell is hypochromatic, with vacuolation of the cytoplasm evident. The nucleolus is pale. ×1200.

role exactly similar to that described in the cerebral cortex (Andrew, 1939*a*). There is a proliferation of the glial satellites about the degenerating cells and a cytolysis of the neuronal cytoplasm, as the satellites 'eat their way' into the nerve cell. Such a degenerate nerve cell, surrounded and in process of destruction by four satellites (in this case, definitely oligodendroglia), is seen in Pl. 1, fig. 5. This is the same process as that demonstrated by us as occurring in starvation in mice, except, of course, that here it is far slower.

From the presence of numerous degenerate, dead, and disappearing motor cells, we must conclude that there is a considerable loss of these cells in animals of advanced age.

The semilunar ganglion

The nerve cells of the semilunar ganglion normally vary a great deal in size. Apart from this, however, we found the appearance of the different cells in any one ganglion to be rather consistently similar.

In the 40-day ♂ and the 41-day ♀ the cells have smooth outlines and large amounts of Nissl material, usually in the form of large blocks or flakes, the appearance and discreteness of which vary somewhat from cell to cell. No pigment is seen in any of the cells. Some of the cells, especially the larger ones, show small, regularly arranged vacuoles in the cytoplasm. The nuclei of the larger cells present the appearance of very clear white vesicles. The nucleoli are conspicuous and deep staining, each one having several large pieces of chromatic material on its periphery. In the smaller cells, multiple nucleoli in one nucleus, up to four in number, are common.

The capsules of the cells in these young animals are but little developed, and, where they are distinct, the nuclei of the capsule cells are of a spheroidal shape. There are small numbers of neuroglial cells between the nerve cells. These cells, however, are not aggregated as 'satellites' and there is no evidence of neuronophagia.

In the 312-day ♂ the cell outlines are smooth and most of the cells have abundant Nissl material. There are no degenerate or 'dead' cells. There is no pigment in any of the cells. A number of the ganglion cells show some vacuolation, rather small vacuoles, of which several dozen can be counted in a section of one cell, appearing in the cytoplasm.

The nuclei of the larger cells are clear, although not so conspicuously so as in the two youngest specimens. Multinucleolism is common in the smallest cells. The nuclei stain fairly deeply and usually each has several distinct basophilic particles on it. The capsules are much more definite, with elongated capsular cells. There is no tendency for satellitosis nor any evidence of neuronophagia.

In the five senile animals the cytological picture is very different. Here the majority of cells are hypochromatic, many having very little or even no Nissl material, although an occasional cell will have as much as is common in the cells of younger animals. A conspicuous feature of almost all of the cells is the presence of large vacuoles in the cytoplasm. These differ from those found in some of the cells of the younger animals both by their much greater size and by the larger proportion of the cytoplasm which they occupy in the aggregate. The larger vacuoles apparently are due to the fusion of the smaller ones. The largest single vacuoles seen occupy a quarter or more of the volume of the cytoplasm.

A yellow-green pigment, of the same appearance as that in the ventral horn cells of the senile animals, is seen here in a small proportion of cells. While in the senile spinal cord this pigment is present in practically every motor cell, in the semilunar ganglion it appears to occur in only about 5 % of the cells.

The nuclei of the cells throughout the ganglion are basophilic. The clear whiteness of the nuclei of many cells in the three younger animals is not seen in any of the cells of the five senile individuals. Some of the nucleoli are very pale and there is a definite decrease in the number and size of the basophilic particles on their periphery. Multinucleolism is still to be seen in the smaller cells.

There are numerous degenerate cells present and a considerable number in which very little of the 'normal' structure of the cell can be made out, only vacuolated, hypochromatic anucleate masses remaining. The amphicytes, cells which are represented in Nissl sections by rather light-staining, vesicular nuclei, either have proliferated about or migrated to the location of these cells, entered within the capsule and are in close apposition or frequently in hollows in the substance of such very degenerate ganglion cells (see Pl. 1, fig. 6). Such pictures strongly suggest a process of neuronophagia.

The Da Fano preparations of the spinal cord and semilunar ganglia, made in the case of the 362-day ♂, the 586-day ♀, and the 696-day ♀ animals, confirm several of the points brought out with the Nissl stain. Thus the cell outlines are more often irregular in the two senile animals than in the one of 'middle-age', the large vacuoles are seen readily in the cytoplasm of the cells of the semilunar ganglion in the old animals, and there are numerous degenerate cells present.

The same technique was used here as in the earlier work (Andrew, 1939*a*), and while the Golgi apparatus was well impregnated in the brain, that in the semilunar ganglion was not brought out well enough to make conclusions justifiable. In the spinal cord the Golgi apparatus was demonstrated fairly well. In the 362-day ♂, it consists of numerous small argentophilic granules about the nucleus, connected by fine threads. In the two senile animals the granules are much coarser and seldom connected.

Further observations on age changes in the brain

The description of the cerebral cortex and the cerebellum of these animals stained by the Nissl method answers well to that given in our previous work (Andrew, 1936, 1937, 1939*a*, 1939*b*, 1940). The age changes include loss of Nissl material, increased basophilic properties of nuclei, and degeneration of some cells in both of these regions of the brain. In the cerebral cortex there is in addition an increased number of glial cells (oligodendroglia and microglia), increased satellitosis, and neuronophagia. In the cerebellum amitotic division of the nucleus with the occurrence of the binucleate condition is common in our seven senile animals.

We wish to point out again (see Andrew, 1939*a*) the presence of an interesting phenomenon to which we have added more convincing instances in the present study: namely, the occurrence of an apparent attempt at actual cytoplasmic division of the binucleate Purkinje cells. Some of the cells of the specimens in the present investigation make such a process seem almost certain.

The Purkinje cells contained pigment in only one senile animal, the 596-day ♀. It was present here only in a small proportion of cells, and only in small amounts in each cell. Pigment is seen definitely in the cells of the cerebral cortex in only two senile animals, the 693-day ♀ and one of the 596-day ♀, that one in which pigment was not found in the cerebellum. Yellow-green pigment was not demonstrated in any parts of the brain in the younger animals.

The dentate nucleus of the cerebellum and the nuclei in the medulla, not investigated for age changes in our earlier work, were studied in all of the animals. The cells in these regions in the young specimens resemble in their abundant Nissl material, frequently clear nuclei, lack of pigment, and scarcity of glial satellites, the cells in other parts of the nervous system. In the senile animals the dentate nucleus shows cells with hypochromatic cell bodies, basophilic nuclei, pale nucleoli, and pigment in a large percentage, or, in some cases, in all of the cells. The nuclei in the medulla show similar changes, with a high incidence of pigmented cells, this incidence running as high as 40–50 % of the cells. Vacuolation of the hypochromatic cells, similar to that seen in the spinal cord, is common. Among the senile animals, satellitosis seems much increased in the medulla and numerous degenerate cells are seen surrounded by glial phagocytes. No binucleate cells are seen in any of the animals in the dentate nucleus nor in the medulla oblongata.

The Da Fano preparations of the brain show a Golgi apparatus in the Purkinje cells and cells of the cerebral cortex of the 362-day mouse consisting of fine granules usually connected by delicate threads. In the senile animals the apparatus consists, as in previous studies (Andrew, 1939*a*, *b*), only of coarse, unconnected granules.

The silver carbonate preparations of frozen sections from one-half of the brain show the astrocytes well in most regions and the oligodendroglia in a smaller number of places, which varied somewhat in each animal. The astrocytes of the mouse brain, both fibrous and protoplasmic, seem similar to those in the brains of other mammals. Sucker feet are numerous. One astrocyte may have several sucker feet, each attached to the same or to a different capillary. The sucker feet vary considerably in length. Some are not much longer than the diameter of the cell body, while others are ten times this length. Most of the longer processes of the protoplasmic astrocytes in the white matter were seen to run parallel to the course of the nerve fibres. There was no observable difference between the astrocytes in young and old animals. There appeared to be no clasmatodendrosis nor any other type of degenerative process in these cells in senility.

The oligodendroglia showed increased numbers and an increased tendency to satellitosis in old age. We do not feel justified as yet in pointing to any morphological distinction between these cells in young and old animals, partly because of their extreme variation in form in each animal. While delicate processes arise from the cell body in most of the cells, it would seem to us that there are numerous cells completely lacking such processes, but possessed of a

relatively large cytoplasmic body, usually in a fan shape, with the nucleus at the narrower end of the body.

Another interesting feature of these cells in our material is their tendency to occur as a symplasm, with two, three, or more nuclei in each cell. This has been reported previously by one other author as a normal occurrence in the olfactory bulb of some animals (Perez, 1933) and as a result of brain lesions in cats (Ferraro & Davidoff, 1928). In our material such cells occurred most frequently in the deeper portions of the cerebrum and in the medulla.

DISCUSSION

In sections stained by the Nissl method we have found definite qualitative differences between the nerve cells of the spinal cord and of the semilunar ganglion of young and senile animals. These differences are large enough to allow one to tell whether a given section is from a young or old animal by low power observation, although the minuter differences require careful study under oil immersion. Our work on the changes in the ventral horn cells in the spinal cord in experimental animals bears out most of the earlier findings on this region in human autopsy material (Nonne, 1899; Sander, 1900; Fluegel, 1927; Stern, 1936). One of the most striking and widespread features in the senile degeneration of these cells is the accumulation of pigment in almost every cell. In our material the irregular vacuolation of the cytoplasm also is conspicuous.

Sander's 'sclerotic' type of degeneration, in which both nucleus and cytoplasm appear light and poorly differentiated, and his type of degeneration in which both nucleus and cytoplasm form a deeply staining mass with indistinguishable Nissl bodies, can be seen in our material. Cells in such states, however, usually show pigment as well, and we feel it questionable as to whether or not these constitute really distinct 'types' of degeneration.

The earlier authors did not stress the relation between the degenerating nerve cells and the interstitial cells. Harms (1924) speaks of numerous 'phagocytes' in the cords of senile dogs. Fluegel describes an increase in glial nuclei in the grey matter in the cords of his six specimens over 80 years of age, but remarks that 'rod cells' were infrequent.

We reported in an earlier paper (Andrew, 1939*b*) an active process of neuronophagia in the brain of starved mice and an apparently gradual one in the brain of normal senile mice. In that paper we spoke of the majority of phagocytic cells as probably microglia, partly because of the classical conception of the role of these cells. In a later paper in which we reported on the same phenomenon in the human brain (Andrew, 1940), we inclined to the view that the phagocytic cells consist of both microglia and oligodendroglia. Our present work, including the silver-carbonate impregnation of the glial cells, has led us to conclude definitely that the majority of cells involved in the process which we described are oligodendroglial cells. This is true of the satellite and

phagocytic cells about the ventral horn cells, as well as in the brain of senile (or starved) animals (see Pl. 1, fig. 5).

The cells of the semilunar ganglion show in senility a rather specific type of vacuogranular degeneration. Pigmentation is much less marked and occurs in only a small percentage of the cells as compared to the spinal cord. It is interesting to note that the amphicytes, which De Castro (1932, p. 97) is inclined to regard as similar to the oligodendroglia, appear to play the same role here as does the oligodendroglia in the spinal cord. They increase in numbers about the more degenerate cells (Pl. 1, fig. 6) and apparently cytolyse and remove them.

Our present observations on the brain have confirmed our earlier work, showed more definite cases of attempts at cytoplasmic division of the Purkinje cells than hitherto seen, and added observations on senile changes in the dentate nucleus and the nuclei of the medulla. The nuclear and cytoplasmic changes observed in these newly studied groups of cells resemble those in the cerebral cortex except for the much greater incidence of pigmentation, which reaches to 50 % or more of the total number of cells. In this respect these cells resemble more the cells of the spinal cord.

In none of the cell-groups considered in the present study, except the Purkinje cells, have we found binucleate cells occurring consistently in senility. This tendency to amitotic division of the nucleus would appear to be a rather specific change for the Purkinje cells of the cerebellum of the rodent.

From the silver-carbonate impregnation studies we may draw several interesting conclusions. Recent work tends to show that clasmatodendrosis can occur in life and not only as a post-mortem phenomenon (Canziani, 1935). We feel that we can say with some assurance, however, that it does not occur as a phenomenon of senile degeneration.

The high degree of polymorphism observed in the oligodendroglial cells would tend to make phagocytic properties of these cells seem more plausible. The ordinary text-book figures and descriptions of these cells, showing a scanty cytoplasm with a few definitely radiating processes, while holding for many of them, certainly give an inadequate conception of the morphology of the group as a whole. Ferraro & Davidoff (1928) pointed out that these cells do act as scavengers and are transformed into compound granular corpuscles, although they did not bring out their relationship to the degenerating nerve cells during this process. The question of the origin of microglial and oligodendroglial cells, notwithstanding the classic work of Rio-Hortega (1921), is by no means settled. While the accepted view is that microglia is probably of mesodermal origin, and hence more 'logically' phagocytic, Rydberg (1932) concludes from observations on both animal and human foetuses that both microglia and oligodendroglia are wholly or predominantly of ectodermal origin.

Cone (1928, p. 67) said: 'Astrocytes and oligodendroglia react in acute disease of the central nervous system by degenerating as nerve cells do. Their changes do not seem to have any obvious purpose....' In senility, we feel, the

reaction of the oligodendroglia is not a degeneration. It is not a regressive, but rather a progressive change. Recent workers (Cardona, 1931) have shown that both types of cell can undergo acute swelling, a reaction supposed formerly to be specific for oligodendroglia.

SUMMARY AND CONCLUSIONS

1. The cells of the trigeminal (semilunar) ganglia and of the ventral horn of the spinal cord show definite qualitative differences between young and senile mice.

2. There are certain changes in the nerve cells in old age common to all parts of the nervous system of the mouse studied thus far. Other changes in senility are more or less specific for different regions:

(*a*) The changes common to all regions are a decrease in Nissl material, increased basophilic properties of the nucleus, increasing paleness of the nucleolus, loss of regularity of cell outline, and degeneration and death of some of the cells.

(*b*) The change most characteristic of the ventral horn cells is pigment accumulation, which is found in senile animals in almost 100 % of the cells.

(*c*) The change most characteristic of the trigeminal ganglion is vacuolar degeneration.

(*d*) An increase of interstitial elements about degenerating cells has been found in all regions except the layer of Purkinje cells. These satellites consist, in the central nervous system, primarily of oligodendroglial cells; and in the ganglia, of amphicytes. The role of such cells seems to be almost undoubtedly phagocytic.

3. The astrocytes of the mouse brain do not show signs of degeneration in senility.

4. The oligodendroglial cells of the mouse brain show considerable polymorphism, many having large fan-shaped bodies, often with very few or no observable processes. The multinucleate condition of these cells is not uncommon. No changes in morphology of the oligodendroglia in senility have been seen, but there is an apparent increase in number and in the tendency to satellitosis.

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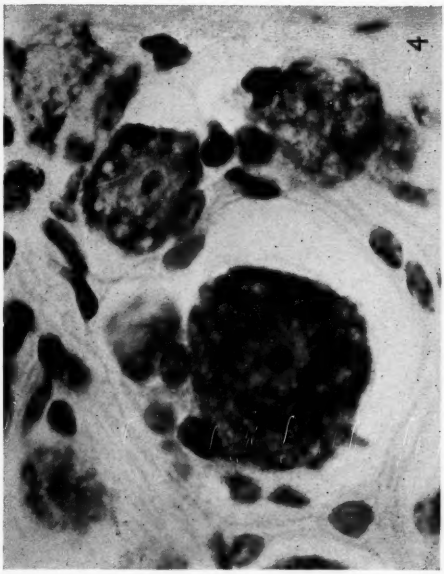
EXPLANATION OF PLATE 1

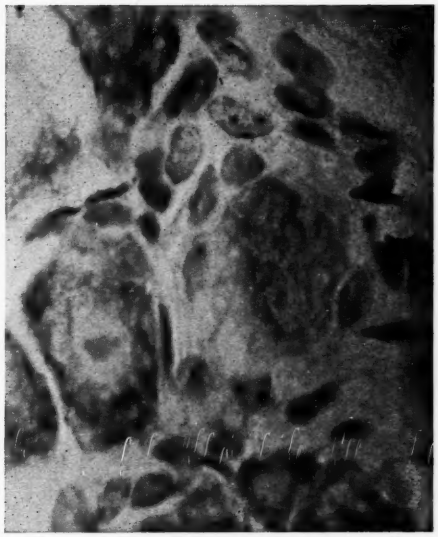
Fig. 3. Cells of the trigeminal ganglion of a young male mouse, 40 days of age. The cells contain abundant Nissl material, and the nuclei of the larger cells are clear, white vesicles. The capsule cells are short, with spheroid nuclei. ×1100.

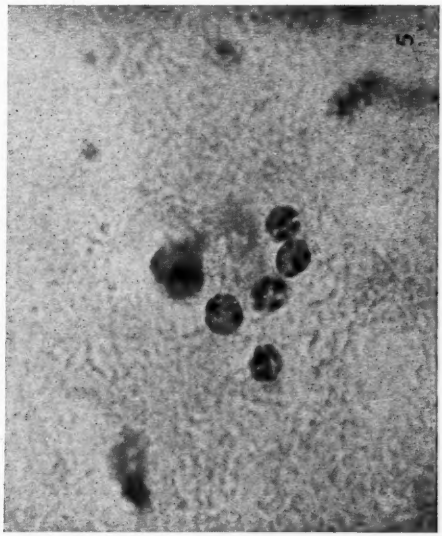
Fig. 4. Cells of the trigeminal ganglion of a senile female mouse, 693 days of age. Nissl stain. The Nissl material is much less abundant, and large vacuoles are conspicuous in the cytoplasm. The nuclei are distinctly basophilic. ×1100.

Fig. 5. Body of an apparently dead motor cell in the ventral horn of a senile female mouse, 596 days of age. The hypochromatic cytoplasm is almost surrounded by glial cells, four oligodendroglial cells and one astrocyte being in contact with it, while another oligodendroglial cell is just below it and to the left. ×1100.

Fig. 6. A degenerate cell from the trigeminal ganglion of a senile female mouse, 595 days of age. The cytoplasm is tattered and shrunken. The nucleus is invisible. An increase in the probably phagocytic amphicytes (clear oval nuclei) is seen. ×1100.







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THE LAMINAR ORGANIZATION AND CELL CONTENT OF THE LATERAL GENICULATE BODY IN THE MONKEY

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The general structure of the lateral geniculate body in the macaque monkey is now well known. However, the precise disposition of its component laminae in a three-dimensional sense is difficult to grasp by reference to sections, even when these are available in several planes. To overcome this difficulty, and in order to facilitate further studies of this visual centre, it was decided to make a wax reconstruction model. The serial sections of the geniculate body were also used for making a computation of its cell content. Further, in order to arrive at an estimate of the number of cells which project on to a unit area of the visual cortex, a preliminary operation had been carried out in which a small lesion of known dimensions was made in the striate area. This led to a circumscribed area of retrograde degeneration in the lateral geniculate body from which it was possible to estimate the number of cells affected.

RECONSTRUCTION MODEL

By way of introduction, a brief reference may be made to the essential structure of the lateral geniculate body in the macaque monkey. The nucleus is composed of six layers of cells arranged in the main dorsi-ventrally, and these, for descriptive purposes, may be numbered in succession from below upwards. Throughout most of the extent of the nucleus as seen in transverse section, the laminae are abruptly arched with a dorsal convexity, being disposed concentrically with regard to an antero-posterior groove or 'hilum' on the ventral surface. The two most superficial (ventral) layers—laminae 1 and 2—are composed of conspicuous large cells whose maximum diameter varies from 28 to 35μ. The other layers—laminae 3, 4, 5 and 6—are composed of smaller cells whose maximum diameter does not usually exceed 20μ. In Nissl preparations only one type of cell is discernible in any one lamina. Crossed retinal fibres terminate in laminae 1, 4 and 6, while uncrossed fibres end in laminae 2, 3 and 5. Serial sections, cut transversely to the line of the optic tract, show that in the whole rostral third of the geniculate body, and at the medial and lateral margins of the middle third, the several laminae appear to merge with each other to a varying extent. Only in the caudal third do all the laminae become topographically quite separated from each other throughout the whole of their transverse extent by intervening fibre layers. Traced in a caudal direction, this separation becomes manifest first in the central region of the

nucleus, then it extends to its medial margin, and lastly the laminae become completely separated up to their lateral extremities.

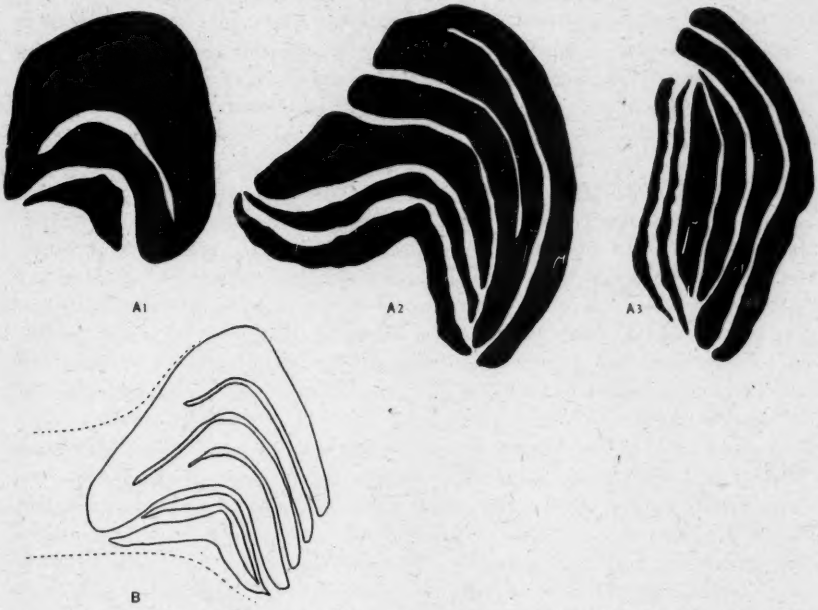
Although in sections of normal material the cells of both the crossed and uncrossed laminae appear to mingle completely where the latter become conjoined, experimental material showing transneuronal degeneration after section of one optic nerve demonstrates that the two sets of laminae, in so far as they are functionally related to crossed and uncrossed retinal fibres, actually remain segregated in these regions. Such preparations show that where the two sets of laminae appear to merge with each other indiscriminately, the cells of each set really remain distinct in separate bands. On the other hand, the three laminae receiving crossed fibres become continuous with each other in front and at the sides the geniculate body, to form a closed system, while another closed and continuous system is formed in the same way by the laminae receiving uncrossed fibres. This structural organization has been represented diagrammatically in a previous publication (Le Gros Clark & Penman, 1934, Text-fig. 1).

For the purpose of reconstruction, use was made of a series of sections through the lateral geniculate body of a macaque monkey (O.M. 449) in which a small cortical lesion had been previously effected (vide infra). A block of tissue containing the lateral geniculate body of the operated side was removed from the brain and, after preliminary hardening in 70% alcohol, was trimmed to a suitable size. Longitudinal grooves were cut on the dorsal and lateral surfaces of the block, to allow of accurate orientation in the reconstruction, and the whole block was embedded in paraffin. Serial sections (10μ in thickness) were cut transversely to the line of the optic tract which runs obliquely backwards and laterally to reach the geniculate body. The sections were stained with Borell's methylene blue. The lateral geniculate body was found to extend from section 43·6 to section 90·3, covering a total of 375 sections and corresponding therefore to an antero-posterior length of 3·75 mm. The outline of the nucleus in every fifth section was traced with a projection apparatus at a magnification of 30 diameters, and the reconstruction model was made by the blotting-paper and beeswax method.

Illustrations of the model from in front and behind are shown in Pl. 1. These should be compared with the drawings of sections shown in Text-fig. 1, in order to appreciate the disposition of the laminae throughout the substance of the geniculate body. As seen from in front (along the axis of the optic tract) the nucleus has a subtriangular form with a rounded apex above. The ventral aspect is marked by a conspicuous hilum. Almost the whole anterior surface is formed by lamina 6 and its fusion with the other small-celled laminae (cf. Text-fig. 1*B*). Below, the most superficial of the large-celled layers (lamina 1) is very evident. Lamina 2 is not visible since it is overlapped by the other laminae. At the medial border, portions of the medial margins of laminae 4 and 5 just come into view.

Seen from behind and slightly from the medial aspect, the model displays

well the arched disposition of the laminae. Most of lamina 1 is visible, lining the concavity of the hilum and sloping abruptly dorsally and medially. It almost entirely overlaps and obscures lamina 2, only the postero-inferior angle of which comes to the surface. The posterior free borders of the four small-celled layers (laminae 3, 4, 5 and 6) are clearly evident, and are here disposed almost vertically. The folding of these laminae, and the manner in which they overlie each other from above and laterally, are more readily apprehended by inspection of the illustration in Pl. 1, fig. 2 than by further verbal description.



Text-fig. 1. A1, A2, A3. Tracings of transverse sections at approximately equal intervals through the antero-posterior extent of the lateral geniculate body. The medial aspect of the nucleus is towards the left. ×10. B. Schematic representation of an oblique section through the median axis of the lateral geniculate body, drawn from a graphic reconstruction. The outline of the optic tract approaching the anterior aspect of the nucleus is shown by interrupted lines. ×10.

The dimensions of the lateral geniculate body as estimated from the model are as follows: antero-posterior diameter (in the axis of the optic tract), 3·75 mm.; maximum width, 4·9 mm.; and maximum height, 4·8 mm.

The model of the human lateral geniculate body constructed by Balado and Franke and illustrated in their monograph (1937) shows that in man the nucleus is different from that of the monkey in being relatively more extended in antero-posterior extent and in transverse width, and more flattened from above downwards. By comparison, in the monkey the whole structure appears

considerably foreshortened and compressed from side to side, so that the cell laminae are much more sharply arched dorsally. It seems, indeed, that the geniculate body of this animal has to accommodate itself within a somewhat restricted space, and that its characteristic shape is at least partly related to the mechanical effects of compression by adjacent parts of the brain.

In Text-fig. 1 is shown a schematic representation of an oblique section through the median axis of the lateral geniculate body, taken along the line of the optic tract; that is to say, obliquely caudally and laterally with respect to the mid-line of the brain. From this diagram it is apparent that at the rostral pole of the geniculate body the small-celled laminae are fused together, while they become separated distinctly by intervening fibre laminae in the middle and caudal part. The diagram also shows the relation of the optic tract to the laminae as it enters the nucleus. Most of the fibres of the tract penetrate directly the conjoined laminae of small cells in a diffuse group of small fasciculi, and many of the latter traverse several laminae before they reach their final destination. Other fibres of the optic tract sweep round the surface of the rostral pole of the geniculate body, and enter the nucleus further back. It is evident that the entrance of the optic tract fibres bears no direct relation to the disposition of the fibre laminae, and, as already pointed out (Glees & Le Gros Clark, 1941), the latter are mainly constituted by the geniculo-cortical fibres. Moreover, the fibres in the optic tract show no segregation or lamination in respect of their origin from the homolateral or the heterolateral eye. This can be demonstrated readily enough in experimental material. In a capuchin monkey (O.M. 466), one optic nerve was sectioned 4 weeks before the animal was killed. The optic tract of one side was sectioned transversely and stained with Bodian's protargol technique. It was found that up to the point where the tract enters the lateral geniculate body, the remaining normal fibres were disposed perfectly evenly throughout the tract, with no tendency to segregation in groups or layers. In other words, it can be inferred that in the normal tract the crossed and uncrossed fibres are intimately and evenly intermingled. It follows, therefore, that the crossed and uncrossed fibres only become disentangled and separated from each other inside the geniculate body.

This arrangement raises an interesting problem in regard to the morphogenesis of the six-layered type of lateral geniculate body. There is evidence that in other afferent systems of the brain localization patterns are impressed upon sensory nuclei by the topographical disposition of the fibres which convey impulses to them (see, for example, Woodburne, 1939). In the case of the lateral geniculate body, this is apparently also the case in regard to the localization within the nucleus of macular and peripheral vision, and in regard to the representation of the upper and lower halves of the retina. But the same principle can hardly be adduced in explanation of the arrangement of the laminae receiving crossed and uncrossed optic fibres. This arrangement, indeed, seems to depend upon an intrinsic differentiation of the lateral geniculate

body—a differentiation which leads *secondarily* to the segregation at this level of the terminals of the crossed and uncrossed fibres.

The laminar differentiation of the lateral geniculate body in the human brain occurs at some time between the fourth and sixth month of intra-uterine development, as shown by specimens in this department. According to Balado & Franke (1937), the first indication of a grouping of cells in the nucleus occurs during the fifth month, but there appear to be no well-defined laminae at this stage, though the optic tract fibres can be seen penetrating into the frontal pole of the nucleus in numerous stout fasciculi and intermingling with the cells. According to our own observations, the lamination is already quite distinct during the sixth month, and by the seventh month (Balado & Franke, 1937; Brugi, 1939) it has acquired the degree of differentiation seen in the adult.

QUANTITATIVE RELATION BETWEEN THE LATERAL GENICULATE BODY AND THE VISUAL CORTEX

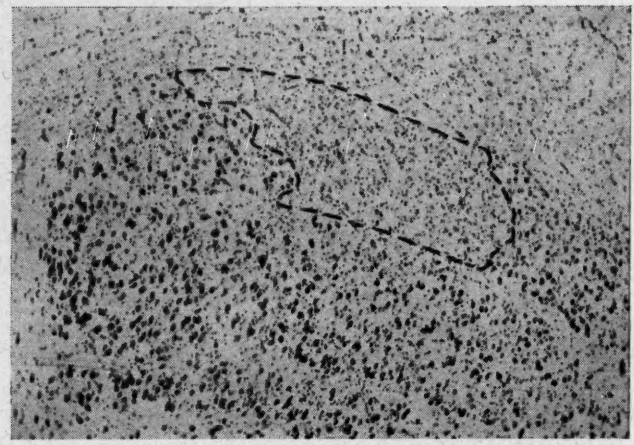
In order to determine the number of cells in the lateral geniculate body which project on to a unit area of visual cortex, use was made of the fact that a circumscribed lesion of the cortex always results in a circumscribed and sharply defined zone of atrophy in the lateral geniculate body. Since the density of the geniculate cells is relatively even at any particular cross-sectional level of the laminae of the geniculate body, it appeared possible, by measuring the area of retrograde degeneration in the sections, to estimate the number of cells which had been affected by a cortical lesion of known dimensions. For this purpose the following experiment was carried out.

In a female macaque monkey (O.M. 449) with a body weight of 3880 g., and in which the permanent dentition was fully erupted with the exception of the third molars, a small lesion was made in the visual cortex. The cortical destruction was effected by the method of devascularization which is commonly employed in this laboratory for small lesions; by this method the lesion is limited precisely to the area of cortex directly involved without apparently affecting the fibres in the subjacent white matter which are connected with neighbouring areas. In this instance, the lesion was produced by means of a cork-borer whose edge had been carefully sharpened. With this instrument a circular piece of pia-arachnoid was cut through, so that the vascular supply to a corresponding area of the cortex was completely interrupted. The site chosen for the lesion was in the anterior part of the striate cortex, immediately behind the sulcus lunatus, and in a region where no large blood vessels were exposed to damage. The slight degree of haemorrhage which resulted from the operation was easily arrested by slight pressure. The whole operation, in which a fairly large exposure was made by turning down an osteoplastic flap, was carried out with the greatest care in order to avoid damage to other parts of the cortex. The animal made a perfect recovery and showed no functional defect. It was killed 33 days after the operation.

Examination of the brain after death showed a perfectly circumscribed lesion of the cortex, 5 mm. in diameter, without any apparent injury to the surrounding cortex. Serial sections taken through the lesion confirmed these observations, and also showed that there was a minimal involvement of the subjacent white matter.

Serial sections, stained with methylene blue, were also prepared through the corresponding area of cortex on the unoperated side of the brain, with a view to a subsequent quantitative study of the cellular content of this part of the visual cortex.

A study of sections of the lateral geniculate body showed that the zone of retrograde cell atrophy is limited to the caudal end of the macular region of



Text-fig. 2. Section of a portion of the lateral geniculate body showing the sharply defined zone of atrophy in lamina 6, following a localized lesion in the visual cortex. ×47.

the nucleus, extending from section 71·2 to section 78·3, and covering therefore an antero-posterior extent of 0·58 mm. In the most rostral sections through the atrophied area, only the deepest of the small-celled laminae (lamina 6) is involved; in more caudal sections the zone of atrophy extends successively through the other laminae from lamina 5 to lamina 1. That is to say, the patch of atrophy involves contiguous parts of all the laminae, as is consistently the case after a destructive lesion of the visual cortex.

In the small-celled laminae the atrophied area is so sharply defined in each section that its margins can be traced out with considerable precision on comparatively low-power projections (Text-fig. 2). A high-power examination of the atrophied area shows that all the nerve cells have here undergone degeneration. The cytoplasm has shrunk to a narrow, poorly staining, perinuclear zone, and the nucleus itself has in most cases dwindled to less than

half its normal diameter. Reference to Pl. 1, fig. 2 makes it evident that no normal cells remain in the area.

In the large-celled laminae, the zone of atrophy is also distinct and localized. Here, however, the boundaries of the zone are not so sharply defined under low-power magnification, and at the margins there is an intermingling of normal and degenerated cells. In the central part of the area, however, all the cells have undergone degeneration (Pl. 1, fig. 4). The cell body has in general shrunk to about two-thirds of its normal diameter, and the nucleus to a somewhat less extent. Moreover, the Nissl granules, though in most cases still present in the cytoplasm, are much finer and more scattered.

In the case of the small-celled laminae, as already noted, the outline of the atrophied zone is sufficiently sharp to allow it to be traced with considerable accuracy. It is thus possible to compute the area of the zone in each section, and therefore to assess its total volume. In order to do this, the outline of every third section of the atrophied area was traced at a magnification of 60 diameters, and the area enclosed by each outline was measured with a planimeter. At this magnification the total area of the atrophied zones amounts to 40,130 mm.^{2} By reducing this to normal size, and taking into account that every third section represents a thickness of 0·03 mm., the actual volume occupied by the atrophied cells in the small-celled laminae was estimated to be 0·334 mm.^{3} In order to determine the density of cells in the affected portions of the laminae, the following procedure was adopted. Photomicrographs, at a magnification of 88 diameters, were taken of the adjacent normal parts of the laminae in eight sections evenly distributed throughout the antero-posterior thickness of the atrophied zone. In each of these photomicrographs, a count was made over as extensive an area as possible in different laminae by pricking out the cells one by one. Only those cells in which a nucleus could be seen were enumerated, and a hand-lens was used to facilitate the counting. Counts were thus made in sixteen different areas, involving the enumeration of 5940 cells, and the size of the areas was subsequently measured with a planimeter. From these data it was found that the average number of cells in an area of 10 cm.^{2} at a magnification of 88 is 94. Reducing this to normal size it can be calculated that the number of cells which would be contained in a volume equivalent to that of the atrophied zone (0·334 mm.^{3}) amounts to 24,342. It thus appears that, as the result of the destruction of an area of visual cortex 5 mm. in diameter ($=19{\cdot}6$ mm.^{2}), approximately 24,000 cells have undergone degeneration in the small-celled laminae of the lateral geniculate body. In other words, each square millimetre of the visual cortex receives from approximately 1220 small geniculate cells the same number of fibres of the optic radiation.

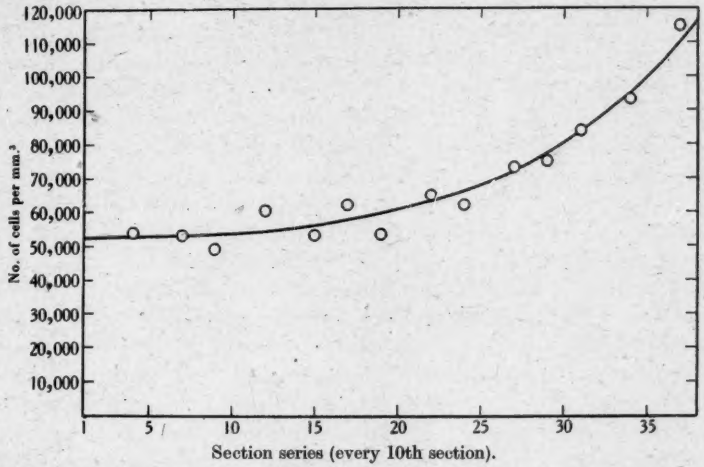
An attempt was made to arrive at a similar computation with regard to the large-celled laminae, but, as already noted, the margins of the atrophied area in these laminae were not sufficiently sharp, and it proved impossible to obtain consistent results.

The same series of sections was used for an estimate of the total cell content of the lateral geniculate body in the monkey. For this purpose, projection drawings of every tenth section of the cell laminae were made at a magnification of 30 diameters (extending from section 44·1 to section 89·6). The areas of the laminae were then measured with a planimeter. Reducing the areas to natural size, the actual volume occupied by the small-celled laminae was found to amount to 24·3 mm.^{3}, and that of the large-celled laminae to 5·1 mm.^{3} To estimate the total cell content of the laminae, it was necessary to ascertain the density of the cells at different transverse levels. As regards the small-celled laminae, the density of cells was found to be of an equivalent value in all the laminae at any one transverse section, but the density increases markedly in a rostro-caudal direction. This is due to the fact that the optic fibres penetrate the rostral pole of the geniculate body in a multitude of small fasciculi so that the cells are here spaced out among them. As the fasciculi become thinned out and dispersed in a caudal direction with the termination at successive levels of the optic fibres in the geniculate body, the cells become more closely packed, and are thus most compactly arranged at the caudal extremity of the nucleus. Photomicrographs at a magnification of 88 diameters were prepared from fourteen sections distributed at approximately regular intervals throughout the antero-posterior extent of the lateral geniculate body. In a number of different areas chosen at random in these photomicrographs, counts of cells were made, involving altogether the enumeration of 7380 small cells and 833 large cells. The areas were measured with a planimeter, and by making the necessary reduction the actual number of cells in 1 mm.^{3} at normal size was calculated. No significant variations in density were found in the large-celled laminae at different transverse levels, related to the fact that these laminae are not appreciably involved by the penetration of the optic fasciculi at the rostral end of the geniculate body. Counts in eleven areas in the photomicrographs showed a range of 43–64 cells for 10 cm.^{2} (σ of distribution $\pm 6{\cdot}3$; mean $53 \pm 1{\cdot}92$). The average number of cells in 1 mm.^{3} in the large-celled laminae at normal size was thus found to be 41,000, and the total number of cells in these laminae calculated to be approximately 209,000.

In regard to the small-celled laminae, the variation in the density of the cells made it necessary to calculate the number of cells per mm.^{3} at the different levels from which photomicrographs were prepared. The values obtained fall on a smooth curve which shows the progressive increase in the cell density through the antero-posterior extent of the small-celled laminae (Text-fig. 3). By reference to this curve, the actual number of cells contained in every ten sections was then calculated. The sum of these figures gave the total number of cells in the small-celled laminae of the whole geniculate body, which was found to be of the order of 1,590,000.

It is interesting to compare this result with the estimated loss of cells in the atrophied area related to the cortical lesion. The total area of the visual cortex in the experimental animal, O.M. 449, was not obtained, since the cortex

of both hemispheres was used for histological examination. However, in another macaque monkey of approximately equal body weight (3980 g.) the area of the visual cortex was measured on both sides of the brain, the average for one hemisphere being 1445 mm.^{2} Applying this figure to O.M. 449, it follows that, if 1220 geniculate cells from the small-celled laminae project on to 1·0 mm.^{2} of visual cortex, then, assuming an even distribution of the fibres of the optic radiation, the total number of cells in the small-celled laminae should amount to 1,760,000, a value which is remarkably close to the number calculated by the more direct method. It suggests that the fibres of the optic radiation are in fact distributed evenly over the whole extent of the visual cortex.



Text-fig. 3. Graph showing the density of cells in the small-celled laminae at different transverse levels throughout the antero-posterior extent of the lateral geniculate body.

Assuming that the cells of the large-celled laminae also project evenly on to the visual cortex, it may be estimated that, with a total of 209,000 cells projecting on to 1445 mm.^{2} of cortex, 145 project on to 1·0 mm.^{2} It thus appears that the total number of optic radiation fibres projecting on to 1·0 mm.^{2} of visual cortex is about 1350.

The quantitative relation between the laminae receiving crossed retinal fibres and those receiving uncrossed fibres could not be determined in regard to the small-celled laminae, for, except in the caudal part of the geniculate body, these fuse with each other to a varying degree at their lateral and medial extremities. The large-celled laminae (laminae 1 and 2), however, are distinct from each other throughout their whole extent, except for a negligible proportion (about 1%) at the most caudal end of the nucleus. In most transverse sections of the lateral geniculate body it is readily apparent that the more superficial of the two large-celled laminae is the wider and more extensive.

As a confirmation of this impression, it was found that the total volume of lamina 1 (receiving crossed fibres) is 3·05 mm.^{3}, corresponding to a cell content of 125,000, while that of lamina 2 (receiving uncrossed fibres) is 1·97 mm.^{3}, corresponding to a cell content of 81,000. Thus, on the assumption that the fibre-cell ratio is of an equivalent order in the two laminae, it may be inferred that, so far as the retinal fibres ending in the large-celled laminae are concerned, approximately 60% are crossed and 40% uncrossed.

Of particular interest is the numerical relation between the cells of the geniculate body and the retinal fibres which make synaptic contact with them. It is not possible to determine this with the available data since, although the number of fibres in the optic nerve of the monkey has been computed at 1,208,000 by Bruesch & Arey (1940), it is not known what proportion of these end in the lateral geniculate body and what proportion pass on to the mid-brain. According to Brouwer (1926), relatively few retinal fibres end in the superior corpus quadrigeminum (in the monkey), and these are more numerous on the crossed side. This judgement, however, is based on Marchi preparations of degenerated fibres which hardly allow of an accurate assessment. Moreover, following recent investigations, account must now be taken of the fact that many mesencephalic fibres of the optic tract (particularly those which mediate the pupillary light reflex) terminate in the pretectal nucleus which lies deep to the superior corpus quadrigeminum (Magoun *et al.* 1936). Direct histological observation has shown that in the monkey each optic fibre ends in relation to 5–6 cells of the small-celled laminae, and there appears to be no overlap (Glees & Le Gros Clark, 1941). If this relation can be accepted as generally valid, then it appears that in each geniculate body of the experimental animal O.M. 449 these laminae would have received approximately 290,000 retinal fibres. The fibre-cell ratio in the large-celled laminae is not known, so that in regard to the latter it is not possible to make a similar computation. In any case, however, it seems not improbable that the proportion of fibres in the optic nerve which are destined for the mid-brain is greater than is generally assumed to be the case in the monkey. The solution to this problem could probably be determined experimentally in the following way. One optic nerve is sectioned in a monkey, and the peripheral stumps removed for the enumeration of the number of fibres in it. After allowing sufficient time for the complete degeneration of the cut fibres, the animal is killed. Counts of the remaining normal nerve fibres are made in both optic tracts. The difference between the total number of fibres in the tracts and the fibres in the cut optic nerve will give the number of non-retinal commissural fibres in each optic tract. Hence the number of crossed retinal fibres in the heterolateral tract and the uncrossed fibres in the homolateral tract can be computed. Since extremely few uncrossed retinal fibres are known to reach the mid-brain, it may be assumed that the great majority of the retinal fibres in the homolateral tract are destined for the lateral geniculate body. Further, a study of the lateral geniculate bodies would allow of the complete differentiation of the laminae

receiving crossed fibres and those receiving uncrossed fibres, by reference to the transneuronal degeneration. This would permit a computation of the number of cells in the two sets of laminae, and thus establish with some precision the fibre-cell ratio in the nucleus.[1]

A comparison of the cell count in the monkey's geniculate body with that given by Balado & Franke for man suggests that in the latter the nucleus is by no means so rich in cells. They give an estimate of 482,664 cells in the small-celled laminae, and 86,181 in the large-celled laminae. They believe, also, that the number of cells corresponds approximately to the number of optic fibres which terminate in the geniculate body, or, in other words, that a one-to-one ratio obtains. They quote the following authorities for estimates of the number of fibres in the human optic nerve: Zwanenburg, 440,000; Kappers, 550,000; and Krause, 400,000. On the other hand, more recent computations by Bruesch & Arey (1940) show that the number may vary from 564,776 to 1,140,030. In any case, however, it seems probable that there are certainly less fibres in the human optic nerve than in the monkey, for the same authors give the figure of 1,208,120 for the latter. In this connexion, it is worth noting the interesting fact that the visual apparatus as a whole may show a more advanced degree of differentiation in the monkey than in man. Woollard (1927) drew attention to the inference that, if visual acuity can be assessed by reference to the degree of intermixture of rods and cones in the macular region of the retina, the density of conducting segments, and the extent and perfection of the fovea, some of the lower primates (e.g. *Cercocebus*) must rank above man. Similarly, it has been pointed out that in the macaque monkey the lamination of the lateral geniculate body is frequently more complicated than in man, for, in the centre of the region which receives macular fibres, lamina 4 may split to allow the passage of a band of cells connecting laminae 3 and 5 (Le Gros Clark & Penman, 1934). Lastly, the visual cortex of several species of monkey certainly shows a higher degree of laminar differentiation.

It is desirable to draw further attention to the cellular atrophy in the geniculate body following a cortical lesion. In the centre of the patches of atrophy in all the laminae, all the cells have undergone degeneration (see Pl. 2). In the large cells of laminae 1 and 2 the degeneration appears not to have proceeded as far as in the other laminae, but this may well be simply related to the larger size of the neurones. Previous experiments in this laboratory in which partial lesions of the visual cortex had been effected have shown similar results, though it is not uncommon to find an occasional cell in the midst of atrophied zones in laminae 1 and 2 which seem to have been more resistant to the process of retrograde degeneration. However, it has

[1] This in fact was the programme of research envisaged when it was decided to carry out the present investigation. Unfortunately, however, when the experimental material had been prepared it was discovered that the monkey whose optic nerve had been sectioned was suffering from an optic neuritis which had led to considerable pathological degeneration of optic fibres. The material had, therefore, to be discarded, and further material is under the present circumstances unobtainable.

been definitely shown by Poliak (1933) that, following total ablation of the occipital lobe in the monkey, the entire cellular content of the corresponding lateral geniculate body undergoes atrophy. There seems no doubt, therefore, that the cells of all the laminae project on to the occipital cortex. But it has been affirmed by Balado & Franke (1937) that the large cells of laminae 1 and 2 have no connexion with the cortex; on the contrary, they believe that their axones join the optic tract and proceed to the superior corpus quadrigeminum where they terminate. They even go so far as to suggest that in the human brain no retinal fibres run directly to the mid-brain, and that those fibres which mediate the pupillary light reflex are relayed in the large-celled laminae of the lateral geniculate body. This evidence appears to rest largely on observations relating to a case of pineal tumour which had led to destruction of the greater part of the superior corpora quadrigemina, and in which histological examination of the brain showed a partial atrophy of the cells of laminae 1 and 2, with an accompanying gliosis. It seems possible, however, that this appearance may have been due to degenerative changes in the mesencephalic fibres of the optic tract which had secondarily and indirectly affected the immediately adjacent cells of lamina 1. Further, their evidence that, in Golgi preparations, the axones of the large cells of lamina 1 can be seen to run downwards to mingle with and join the fibres of the optic tract does not conclusively establish that they proceed with the tract to the mid-brain, for they may skirt the margin of the geniculate body to join the optic radiations on their way to the cortex. Altogether, indeed, the evidence adduced by Balado & Franke seems hardly sufficient to outweigh, or even to balance, the striking evidence of the retrograde atrophy which affects the large-celled laminae after cortical lesions. In other words, it may be accepted (unless further evidence to the contrary becomes available) that these large cells, like the small cells of the geniculate body, project on to the visual cortex. The morphological difference between the large and small cells thus remains unexplained, though the assumption is no doubt justified that it is related to a functional difference of some kind. Such a conclusion also seems to be indicated by the fact that transneuronal atrophy does not proceed so rapidly in the large-celled as in the small-celled laminae, following section of the optic nerve.

On the other hand, it should be noted that both the large and small-celled laminae appear to have an equivalent value in regard to different parts of the retina (central or peripheral) and also in the sense that both types of laminae project on to all parts of the visual cortex. For it has been demonstrated (Le Gros Clark & Penman, 1934) that small and localized unilateral retinal lesions consistently lead to a transneuronal atrophy involving two small-celled laminae and one of the large-celled laminae, while Poliak (1933) has also established the fact that small and localized lesions in different parts of the visual cortex consistently lead to a retrograde degeneration involving all six laminae.

It does not appear that the large size of the cells in laminae 1 and 2 is related to a greater thickness of their axones, for in silver preparations the

latter show no conspicuous difference in this respect from the axones of the small cells of the other laminae. But it is difficult to make certain of this, since the axones from all the layers of the geniculate body become closely intermingled in the optic radiations. On the other hand, the dendrites of the large cells are stouter, more numerous, and more extensive. Silver sections through the optic tract of the monkey show that the retinal fibres vary in calibre to some degree, and it may be that the thicker fibres are specifically related in their termination to the large-celled laminae. In regard to this point again, however, it has not been possible to establish any conclusion. Our computations show that in the monkey the number of large cells is between one-seventh and one-eighth that of the small cells, while in man the figures of Balado & Franke show a proportion of between one-fifth and one-sixth. If the large- and small-celled laminae receive proportionately an equivalent number of optic fibres and are related to different types of retinal element, it is to be presumed that the latter would bear a similar numerical relation to each other. It is tempting to consider whether there may be any definite relation between the large diffuse ganglion cells and the midget individual ganglion cells of the retina (which have been described by Poliak, 1936) and the large- and small-celled laminae, for Poliak has noted that the large ganglion cells are numerically much fewer than the midget cells in the monkey. However, it is difficult to know how the possibility of such a relationship might be investigated.

From the point of view of comparative anatomy, it may be noted that the full development of the large-celled element is a characteristic feature of the higher primates, for, although in lower forms laminae with cells of different sizes certainly occur in the lateral geniculate body, it is only in monkeys, apes and man that the large and small cells are so sharply segregated into different laminae, or that the two categories of cells show so obtrusive a contrast in their size.

The observation that all the cells of the lateral geniculate body of the monkey undergo degeneration following ablation of the occipital lobe is strong evidence against the existence of intercalated, short-axone elements in this nucleus. A similar conclusion is indicated by the fact that Nissl preparations show not more than one morphological type of cell in each lamina. Nevertheless, intercalated elements have been described (on the basis of Golgi preparations) in the cat by O'Leary (1940), and in man by Henschen (1925). However, according to Taboada (1927), who made a detailed study of the cytoarchitecture of the monkey's geniculate body, the diverse types of cell described by Henschen really represent different varieties of a single type, the differences in appearance being entirely due to the topographical position in which each cell finds itself. The same author also established that, in the monkey, the dendritic processes of the geniculate cells are confined in their distribution to the individual lamina in which the cells are situated.

SUMMARY

1. A wax reconstruction model of the lateral geniculate body of the monkey is described and figured.

2. The crossed and uncrossed fibres of the optic tract do not become segregated until after they have penetrated the lateral geniculate body.

3. Computations of the cell content of the lateral geniculate body have been made by a direct method of counting, and also by estimating the number of cells involved in retrograde atrophy following a localized cortical lesion of known dimensions. The number of cells in the small-celled laminae is assessed at approximately 1,590,000, and in the large-celled laminae 209,000.

4. Approximately 1850 fibres of the optic radiation project on to each square millimetre of the visual cortex.

5. Evidence is adduced that, so far as the retinal fibres ending in the large-celled laminae are concerned, approximately 60% are crossed and 40% uncrossed.

6. All the cells of the large- and small-celled laminae project on to the visual cortex, and no evidence is found for the existence of intercalated neurones in the nucleus.

I wish to thank Mr E. Thompson for his technical assistance in the preparation of serial sections, Dr Eva Glees for help in recording the planimetric data, and Mr W. Chesterman for the great care he has taken in the preparation of the reconstruction model.

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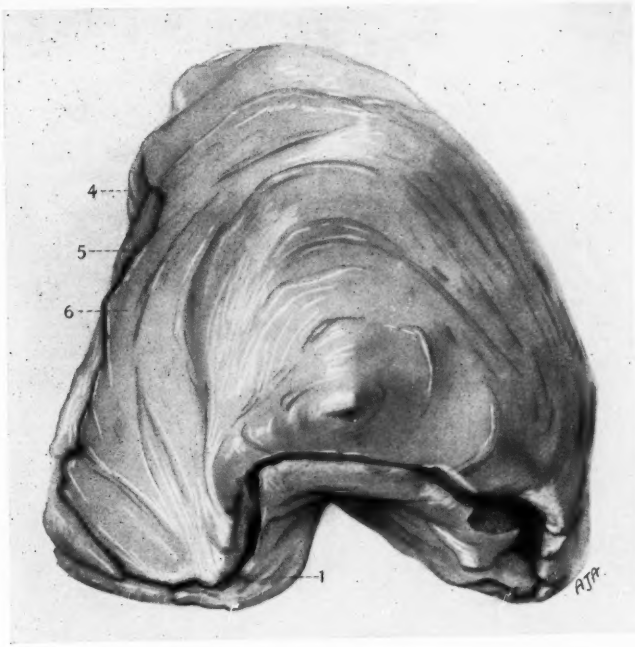

Fig. 1.

Fig. 2.

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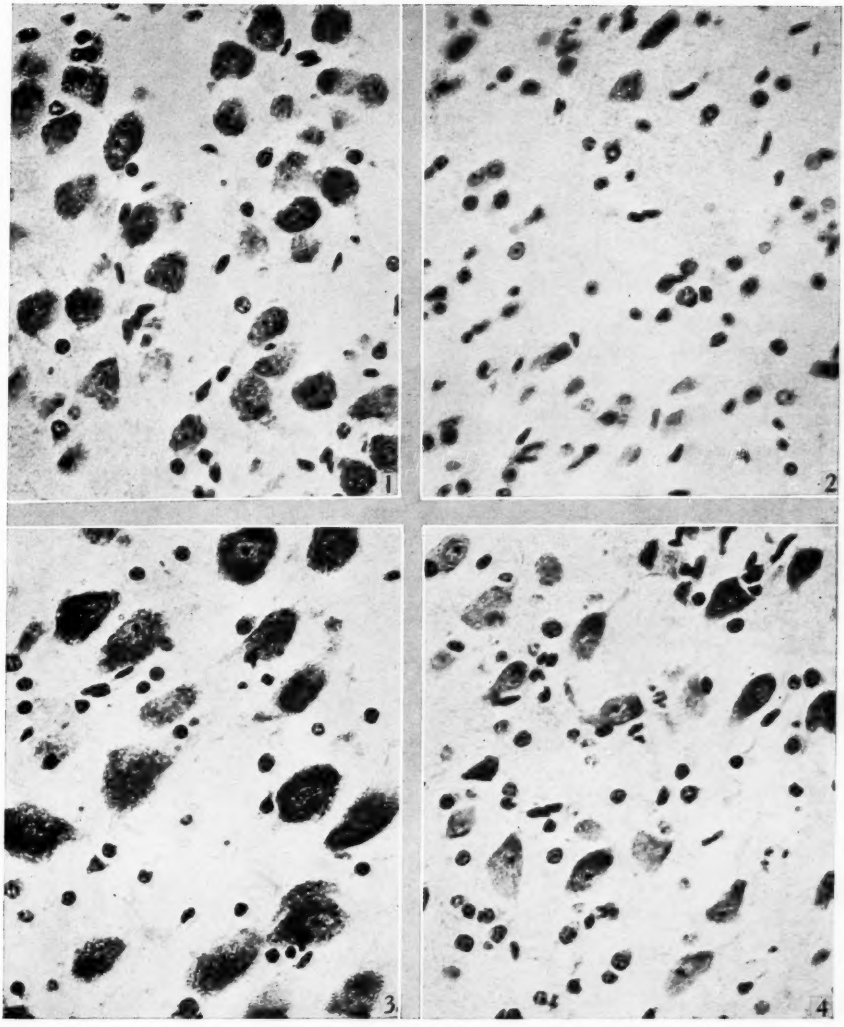

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EXPLANATION OF PLATES 1 AND 2

Plate 1

Fig. 1. Reconstruction model of the left lateral geniculate body of a monkey seen from in front (along the axis of the optic tract). The numbers refer to the various cell laminae (see text).

Fig. 2. Reconstruction model of the left lateral geniculate body seen from behind and slightly from the medial aspect (with reference to the axis of the optic tract). The numbers refer to the various cell laminae. Both figures show the model at approximately three-fifths of its size, that is, about 18 times the size of the actual nucleus.

Plate 2

Fig. 1. Normal cells of one of the small-celled laminae of the lateral geniculate body. ×390.

Fig. 2. Atrophied zone in one of the small-celled laminae showing retrograde degeneration 33 days after a lesion in the visual cortex. No normal cells are now present. ×390.

Fig. 3. Normal cells of one of the large-celled laminae of the lateral geniculate body. ×390.

Fig. 4. Atrophied zone in one of the large-celled laminae showing retrograde degeneration 33 days after a lesion in the visual cortex. All the cells are considerably shrunken, and no normal cells persist.

THE TERMINATION OF OPTIC FIBRES IN THE LATERAL GENICULATE BODY OF THE CAT

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In a recent paper (Glees & Le Gros Clark, 1941) attention was drawn to the mode of termination of the optic fibres in the lateral geniculate body of the monkey. It was shown that in normal preparations of the geniculate body terminal boutons (though relatively very few in number) can be demonstrated in relation to the cells. In order to determine whether these boutons were of retinal origin the optic nerve was sectioned with a view to studying any degenerative changes which they might subsequently undergo. These experiments showed, in fact, that following such an operation the terminal boutons undergo characteristic changes. Three days after sectioning the optic nerve a densely stained neurofibrillar substance appears in the annular end-formations, while occasionally they become converted into solid black bulbs. After seven days the annular types of ending are only seldom to be observed, and even these are not of normal appearance. By taking advantage of the degenerative changes it was possible to stain the optic fibres selectively. From such material it appeared that the optic fibres turn out at right angles from the fasciculi of the optic tract in order to enter their appropriate cell laminae. In the latter, each optic fibre divides into five to six branches and these were seen to end in darkly stained swellings. Each degenerated end-bulb was found to lie in contact with one geniculate cell. It was concluded from these experiments that the end-swelling is the pathological equivalent of the terminal annular formation seen in normal preparations. The experiments further suggested that each optic fibre ends in relation to five to six cells, and, since it was not possible to demonstrate more than one bouton in relation to each cell, it seemed probable that no overlapping occurs in the geniculate body of the monkey.

The present paper is a report of a similar neurohistological study on the geniculate body of the cat. This investigation has been greatly facilitated by the recent work of O'Leary (1940) based on Golgi preparations of foetal material, for this careful work has provided the necessary embryological foundation for a study of the adult structure.

According to Tello, Cajal, and O'Leary, all of whom worked on Golgi material, the optic fibres of the cat end in the geniculate body in brush-like and free terminals. Minkowski (1920) and Barris (1935) have established that crossed and uncrossed retinal fibres end in different cell layers, while the projection of the retinal quadrants on each geniculate body has been studied by

Overbosch (1927). As already indicated in the excellent work of Thuma (1928), the conclusions of Minkowski and Overbosch regarding the localization of optic terminals in the geniculate body were not completely in accord. The differences are to be explained by the different methods employed. Minkowski found crossed and uncrossed fibres ending in sharply defined cell laminae and he was able to demonstrate this by reference to the phenomenon of trans-neuronal degeneration. On the other hand, Overbosch based his results on the study of Marchi degeneration following small lesions in the retina and did not concern himself with the relation of the degenerated fibres to the cell layers. The object of the present work is mainly a neurohistological study of the ultimate endings of the optic fibres in the normal geniculate body and of their reaction following section of the optic nerve.

MATERIAL AND TECHNIQUE

For both the normal and experimental material full-grown cats were used. In all cases the animals were killed by an overdose of ether and immediately perfused with 10 % formol saline. In the experimental animals the left optic nerve was sectioned at intervals respectively of 3, 5 and 7 days before death. The silver technique employed was the same as that previously used in the study of the monkey's geniculate body. The Hortega method was found particularly suitable for the cat material, and for this reason was mainly used.

NORMAL HISTOLOGY OF THE GENICULATE BODY

In the following description attention will be given only to those histological features which are of importance for the present study. This limitation is justified by the work of previous authors dealing with the cell and fibre architecture of the lateral geniculate body of the cat. Our preparations confirm an earlier observation by Barris *et al.* (1935) that the majority of the crossed optic fibres in the geniculate body are collaterals which leave the optic tract at right angles, the main fibres proceeding further distally to reach the tectum opticum. The collateral fibres enter the geniculate body and ramify among its cells. The lamination of the geniculate body is most clearly evident in sagittal sections and it was found that the terminal branchings of the optic fibres are also most clearly seen in this plane. Thuma has already emphasized the difficulties of recognizing the nature of the cell lamination in transverse sections. His illustrations and descriptions have been found of the greatest value in the present study.

The optic fibres ramify with great profusion among the geniculate cells, and with the dendrites of the latter they form an extremely dense plexus. It has already been noted that according to Tello, Cajal, and O'Leary the optic fibres in the geniculate body terminate in free endings. However, with

appropriate staining technique and with the use of an oil-immersion lens, the geniculate body in the cat can be seen to be strewn with fine terminal rings of characteristic appearance. In order to avoid any possible error of interpretation, sections of the spinal cord were prepared with the same technique. In this material the terminal boutons in relation to the anterior horn cells were stained very clearly, and in appearance were quite similar to the fine terminal rings seen in the geniculate body. Only very occasionally was it possible to observe in normal preparations a connexion between the terminal rings and the fine terminal ramifications of the optic fibres. The boutons lie in contact with the dendrites and the perikaryon of the geniculate cells. In some cases the impression is gained that the interior of the fine rings is not optically empty but is filled with a fine granular substance.

Degenerative changes in the geniculate body following section of one optic nerve

Exp. 1, 3 *days.* By careful impregnation of the sections, and by avoiding excessive reduction of the silver, it was found possible selectively to stain the degenerating fibres and thus to make them stand out conspicuously among the other fibre components of the lateral geniculate body. In such preparations deeply stained fibres can be seen emerging from the optic tract. Within the geniculate body the fibres divide at first into two branches of equal calibre and these further break up into numerous ramifications. The finest of the small branches are seen to end in solid black bulbs and it is apparent that a great proportion of the fine terminal rings of the normal geniculate body have become converted into these deeply staining bulbs. Where the annular formation is still maintained its outline appears much more sharply defined and it is also thicker than in normal preparations. Besides these degenerative changes some terminal rings are to be found here and there which do not appear to differ from normal.

The degenerated endings of the optic fibres lie in contact with the dendrites and cell bodies of the geniculate cells. The appearance in the sections gives the strong impression that the axo-dendritic contact preponderates numerically over the axo-somatic.

Whether the perikaryon is brought into relation with more than one optic fibre is difficult to determine. It seems probable, however, from a consideration of the relative length of the dendritic processes that each cell may ultimately be in synaptic relationship with more than one optic fibre.

Exp. 2, 5 *days.* At this stage of degeneration it has become more difficult to demonstrate a complete picture of the mode of branching of each optic fibre, for it appears that the staining property of the fibres is diminishing. The terminal ramifications are seen to be in all phases of degeneration. There are still present many fine fibres ending in densely staining rings. The solid end-bulbs, however, do not stain such an intense black as in the 3-day experiment

and many of them are beginning to disintegrate into small granules. In addition there are also to be seen oval or elongated end-formations in the substance of which a strongly staining neurofibrillar network is visible. A study of the 5-day stage provides further information on the question of the synaptic relation between optic fibres and geniculate cells. For example, in one instance it was possible to follow the terminal ramifications of a single optic fibre as far as their contact with the perikaryon of two neighbouring cells, while other branches of the same fibre could be traced up to the dendrites of these cells.

Since in this 5-day stage the cells (with their processes) and the end-formations of the optic fibres are in many sections quite clearly stained, it was possible to make an approximate estimate of the numbers of synaptic contacts related to one cell. Thus it was found that the medium-sized cells each make about forty axo-somatic and axo-dendritic contacts with the terminal boutons of the optic fibres. As in the previous experiment, it is difficult to determine whether these synapses are derived ultimately from one or several optic fibres, but since the dendritic processes extend over considerable distances it seems probable that contact is in fact established with more than one optic fibre.

Exp. 3, *7 days.* The optic fibres entering the geniculate body are now beginning to undergo fragmentation, so that it is no longer possible to demonstrate the terminal branching of individual fibres. Fine terminal rings can also no more be found in connexion with the degenerated optic fibres. However, such connexions may still be present, but obscured, for it is still possible in the degenerated areas of the geniculate body to detect apparently normal ring-shaped boutons. Many of the end-formations are still evident as solid bulbs, but a great proportion of these seem to have undergone disintegration and appear as granular debris.

DISCUSSION

The observations recorded above make it clear that the optic fibres do not terminate as free endings in the lateral geniculate body of the cat, but that they effect synaptic contacts with the geniculate cells by means of end-feet or boutons. This conclusion appears at first sight to be at variance with the findings reported by Tello, Cajal, and O'Leary. But this apparent discrepancy is readily explained by the different histological techniques employed and also by the fact that in the present study mature cats were used. As already pointed out by Windle and Clark in their study of the morphology of the synapse (1928), no specific end-apparatus or boutons are to be found in new-born animals. These boutons become first differentiated with the progressive maturation of the tissues of the central nervous system. The Golgi technique is in most cases applied to foetal material, but the absence of end-feet in such preparations is not only due to the age of the animal—the technique itself is not adequate for the detection of boutons which can only be seen with the highest magnifications.

The presence of fine ring-shaped boutons on the terminal branches of the optic fibres in relation to the geniculate cells shows that, as in the geniculate body of the monkey, these fibres have specific endings in the cat. It is to be hoped that, with further study and with further refinements of histological technique, those regions of the brain, such as the cerebral cortex, in which only free endings are commonly believed to occur, will become more and more restricted; for such specific endings (boutons or end-feet) provide a welcome opportunity for the quantitative and qualitative analysis of synaptic relations. Anyone who studies preparations of normal silver material will recognize how impossible it is, in the midst of a dense neuropil such as exists in the lateral geniculate body, to determine whether this or that fibre really ends in actual contact with the cells topographically related to it.

The experimental material has provided evidence that the boutons in the cat's geniculate body, together with the optic fibres themselves, undergo a characteristic degeneration after section of the optic nerve. These preparations also offer confirmation of some of the observations recorded by O'Leary. Thus, each principal cell makes a considerable number of synaptic contacts with optic fibres—in some instances approximately forty boutons have been counted. Further, a single optic fibre supplies more than one geniculate cell, and it is probable that as many as ten cells can be in contact with the terminal arborizations of one fibre. Lastly, our preparations strongly suggest (though they do not conclusively prove) that individual geniculate cells make synaptic contacts with the branches of more than one optic fibre; in other words, there is an overlap in the termination of neighbouring fibres in the geniculate body.

It is interesting to compare these results with the conditions which obtain in the monkey. In the latter it was found that the terminal boutons are extremely few in number, and since it was in no case possible to find more than one bouton related to each geniculate cell, it was concluded that in the lateral geniculate body of this animal there is no overlap of optic fibres. Clearly, such an arrangement would provide for the utmost precision in the recording at this neural level of a retinal image. On the other hand, the profusion of terminal boutons in the geniculate body of the cat, together with the overlap which appears to exist, would provide an anatomical basis for a high degree of sensitivity in low intensities of illumination.

Attention may be drawn to the fact that, as these experiments show, the endings of fibres can undergo very rapid degeneration following section of the main fasciculi from which they arise. This phenomenon can be made evident not only by the use of a specialized silver technique, for Meader (1939) found with the application of the Marchi technique that four days after the enucleation of one eye in a rabbit the degeneration of the myelin sheath of the optic fibres is already evident in the terminal optic centres. On the other hand, Marchi degeneration in the proximal end of the optic nerve can be first demonstrated only after $7\frac{1}{2}$ days.

There are a number of other problems relating to the geniculate body of the cat for which a solution may perhaps be found by the application of the technique used in the present study. For example, it is still uncertain whether any optic fibres terminate in the ventral nucleus of the lateral geniculate body. It is true that O'Leary has quite lately reaffirmed the existence of such connexions, but his evidence was based on Golgi preparations of normal material, and it has yet to be proved that the fine fibres seen entering the ventral nucleus actually have their origin in the retina. For it is a fact that the optic tract, at the point where it reaches the geniculate body, incorporates fibre systems which have nothing to do with the visual fibres (see in this connexion a recent paper by Jefferson, 1940). Other questions which merit attention are (1) a confirmation by the silver method of the conclusion (based on the phenomenon of transneuronal degeneration) that crossed and uncrossed optic fibres end in different cell laminae; (2) whether the geniculate cells of the different sizes which have been so beautifully demonstrated by O'Leary show equivalent quantitative or qualitative differences in their synaptic relations; and (3) whether the geniculate cells receive other afferent fibres with terminal rings besides those of retinal origin. That the last question may well be answered in the affirmative is suggested by the persistence of apparently normal rings in the midst of the degenerated zone in the lateral geniculate body after section of one optic nerve.

SUMMARY

1. It has been found in the cat that the optic fibres arising as collaterals from the optic tract terminate in the lateral geniculate body in special end-formations, i.e. fine terminal rings.

2. After section of an optic nerve these optic terminals undergo a characteristic degeneration.

3. The number of synaptic contacts of optic terminals with one principal cell of the geniculate body is estimated to be about forty.

4. Synaptic contacts are established with the dendrites as well as with the cell body. The axo-dendritic contacts are more numerous than the axo-somatic.

5. The evidence suggests that one optic fibre covers with its terminal branches an area containing about ten cells. It is almost certain, however, that the same region receives a supply from more than one optic fibre and that there is an extensive overlap.

I wish to thank Prof. Le Gros Clark for carrying out the operative procedure necessary for the experimental material and for assistance in the preparation of this paper. I gratefully acknowledge also a grant from the Nuffield Committee for the Advancement of Medicine.

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EXPLANATION OF PLATE 1

Fig. 1. *Exp.* 1, 3 *days.* Below, a branching optic fibre is seen in the first stage of degeneration. At the top of the picture and slightly to the right is seen a degenerated bouton. ×820.

Fig. 2. *Exp.* 3, 7 *days.* Thickened, degenerating optic terminal fibres are seen embracing a geniculate cell. ×2000.

Fig. 3. Two normal, ring-shaped boutons are seen lying in contact with dendrites of geniculate cells. ×2000.

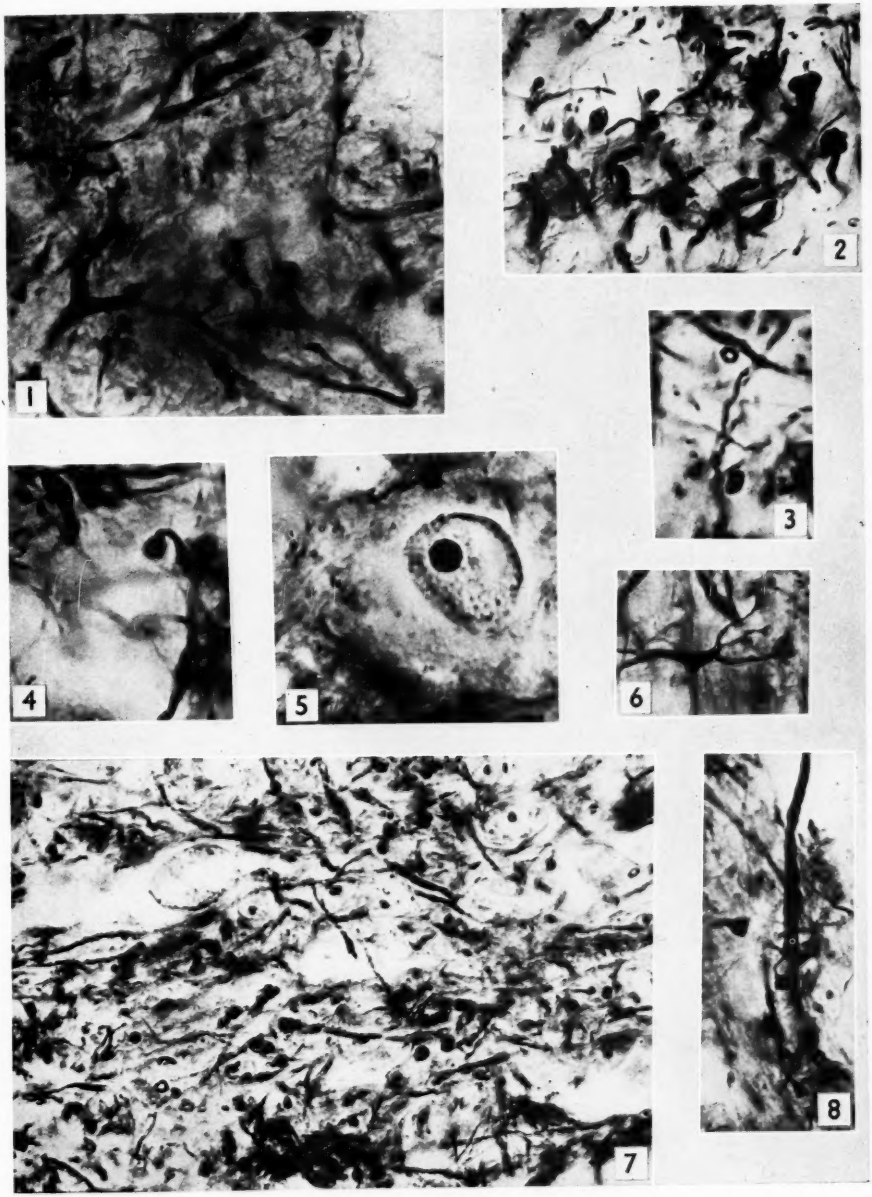
Fig. 4. *Exp.* 1, 3 *days.* A degenerated bouton is seen lying in contact with the cell body of a geniculate cell. The position of the latter is indicated by the clear space. ×820.

Fig. 5. *Exp.* 1, 3 *days.* A slightly degenerated bouton is seen lying in contact with the upper margin of the cell body of a geniculate cell. ×2000.

Fig. 6. *Exp.* 1, 3 *days.* A branch of an optic fibre is seen dividing into three terminal ramifications. The middle one is only very slightly degenerated. The outer two show different degrees of more advanced degeneration. Note that all these three ramifications take origin from one main branch and that the site of branching is thickened. ×820.

Fig. 7. *Exp.* 2, 5 *days.* Numerous boutons are seen scattered over the field in different phases of degeneration. Note an apparently normal bouton towards the lower left-hand corner. ×820.

Fig. 8. *Exp.* 1, 3 *days.* Several boutons are seen lying in contact with a dendrite. The proximal end of the dendrite joins the cell body towards the lower right-hand corner of the photomicrograph. ×820.



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THE MULTIPLE INNERVATION OF SENSORY SPOTS IN THE SKIN

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The conception that each single sensory spot in the skin is innervated by more than one nerve fibre is by no means new, but until quite recently it has been based entirely on theoretical considerations. These theoretical considerations have, however, not always been the same. Thus Head & Rivers (1908) postulated an innervation by two different sets of fibres of different phylogenetic significance and with different rates of regeneration to account for their dual forms of protopathic and epicritic sensibility. Boring (1916), on the other hand, was led to infer a multiple innervation of sensory spots by fibres of a common type to provide an anatomical basis for his own observations on the cutaneous sensory phenomena which follow the section of a peripheral nerve and which occur during the process of regeneration, and particularly to explain his ideas of central inhibition.

The employment of the technique of vital staining with methylene blue has permitted an approach to this problem from the anatomical point of view, for by this method it is possible to examine relatively large stretches of skin and subcutaneous tissue and so to follow individual nerve fibres in continuity for considerable distances to their termination in end-organs or terminal nets. By taking advantage of this opportunity it has lately been demonstrated that each single group of hair follicles in the ear of the rabbit is innervated by two or more fibres which approach their destination from different directions, and that a cold spot in the human skin in the same way overlies two or more groups of Krause's end-bulbs receiving fibres which approach them from opposite directions (Weddell, 1941).

The question now arises whether a similar disposition obtains with Meissner's tactile corpuscles. These specialized endings do not occur in the skin of the rabbit, but, by good fortune, it has recently been possible to study their topographical arrangement in the skin of a human finger-pad.

Case report. A patient (D. B.) developed a Dupuytren's contracture involving the fourth finger of the right hand. The contracture was causing sufficient disability to make amputation of the finger at the metacarpophalangeal joint necessary. The skin over the pad of the finger appeared somewhat thinner than usual, presumably owing to the enforced disuse which the contracture had caused. However, tactile sensibility as estimated with a von Frey hair was similar both qualitatively and quantitatively to that over the pad of the opposite normal finger. The limen of two-point discrimination for touch was of the order of 1·5 mm. over both fingers.

Histological technique. Immediately before the amputation of the finger the skin of the pad was infiltrated with a 0·03 % solution of B.D.H. standard methylene blue stain in 0·9 % sodium chloride A.R. During the operation a tourniquet was applied to the arm, causing complete arrest of the circulation. It was released some minutes after the injection of the dye and the skin removed from the finger 15 min. later. The skin was placed in an 8 % solution of ammonium molybdate in which it was allowed to remain for 24 hr. It was then washed, dehydrated, cleared and first examined as a whole preparation. Razor slices were made subsequently to allow of the more critical examination of selected areas.

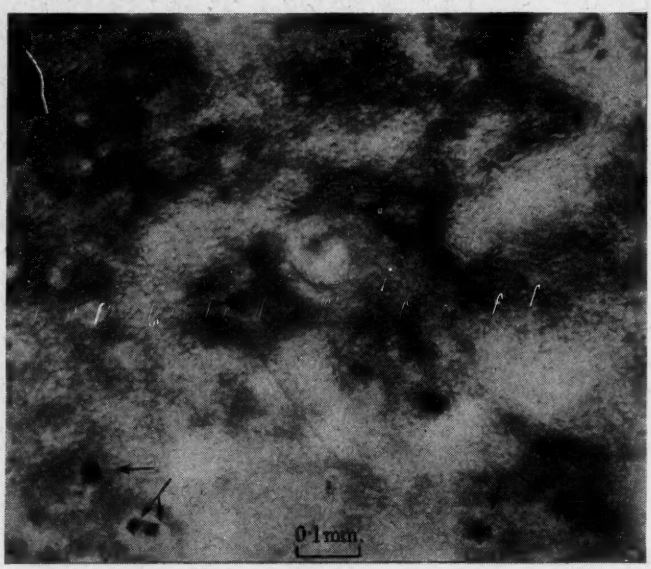

Fig. 1. This shows groups of Meissner's corpuscles in the finger pad of the fourth finger from a human subject. The arrows indicate the position of Meissner's corpuscles in different groups. Owing to the varying thickness of the skin surface not all the end organs are clearly in focus in this one photograph. Methylene blue preparation.

Histological observations. Fig. 1 is a photomicrograph taken through the superficial surface of the skin. It shows groups of Meissner's corpuscles lying in dermal papillae. Altogether there are three groups of two corpuscles and one group of three corpuscles. When traced more deeply beneath the skin, the corpuscles are found to lie in the long axis of the dermal papillae which are at times obliquely situated with regard to the skin surface. Occasionally there are two corpuscles within a single papilla. It is estimated from a number of counts from different areas that there are approximately ten groups of Meissner's corpuscles per sq. mm. in the finger pad of the fourth finger, and that each

group consists of two or three separate end-organs. In a piece of skin taken more towards the dorsum of the distal phalanx the number of groups of corpuscles is found to be diminished; each group, however, still consists of two or three end-organs. No solitary Meissner's corpuscles are seen.

Fig. 2 is a scale drawing which shows the disposition of three contiguous Meissner's corpuscles as seen from the deep surface of the skin. They are situated within an area of which the greatest diameter is 0·15 mm. It is probable that

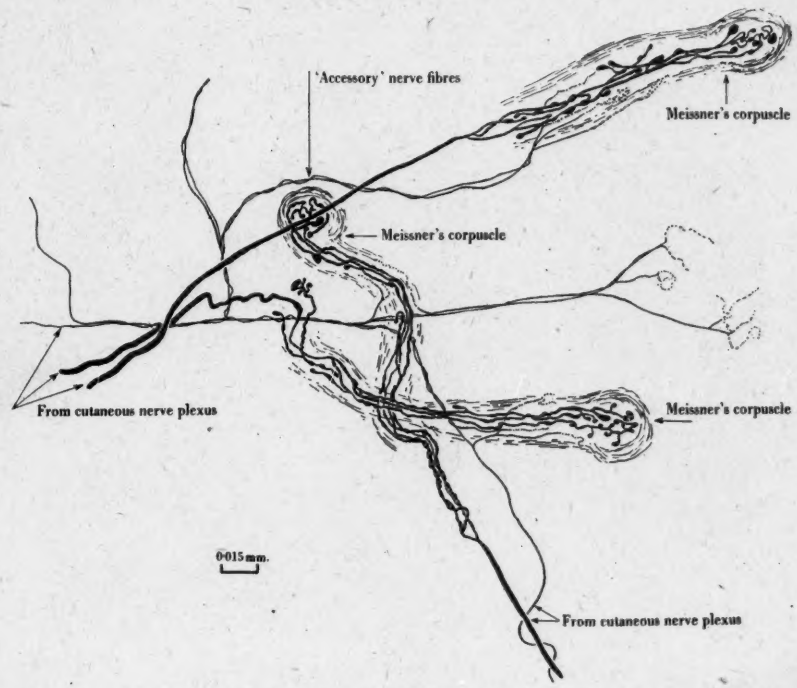

Fig. 2. A scale drawing showing three closely related Meissner's corpuscles each of which is borne upon a separate nerve fibre. Fine unmyelinated nerve fibres giving rise to beaded terminals which ramify in the connective tissue capsule of the Meissner's corpuscles are also shown.

tissue shrinkage and flattening of the skin during fixation have exaggerated the horizontal lie of the corpuscles, but their relative positions remain as in life. Each corpuscle is borne upon a separate myelinated nerve fibre which can be traced for some distance through the cutaneous nerve plexus, where it pursues a simple course without branching. Fig. 3 is a photomicrograph showing details of the neural portion of one of the Meissner's corpuscles as seen in a somewhat oblique plane. The medullated nerve fibre to each corpuscle is accompanied by

a non-medullated 'accessory' fibre which ends in fine beaded terminals in the capsule surrounding each corpuscle (Fig. 2).

More deeply situated beneath the epidermis, there is a cutaneous nerve plexus consisting of medullated fibres of various diameters and fine non-medullated fibres. The thick medullated fibres undergo no dichotomization and give rise to no branches. They all end in Meissner's corpuscles. In two places smaller medullated fibres are seen to leave the cutaneous nerve plexus in which they have remained single and give rise to five and six branches respectively; each of these bears an ending which resembles a Krause's end-bulb. The finer nerve fibres undergo repeated dichotomization within the nerve plexus; many are seen to leave and give rise to small superficial nerve nets, which in turn give rise to fine beaded terminals scattered beneath and between the cells of the deeper layers of the epidermis.

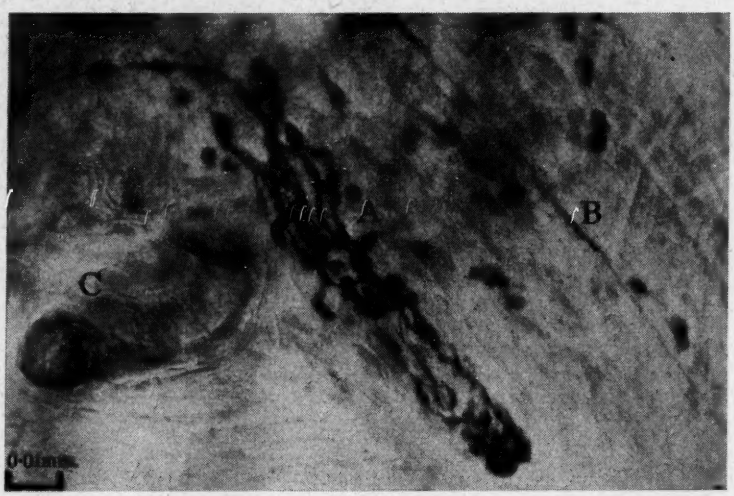

Fig. 3. A Meissner's corpuscle from the pad of the fourth finger of a human subject, showing the complex branching and leaf-like expansions of its neural elements. Methylene blue preparation. A, Meissner's corpuscles; B, neighbouring unmyelinated nerve fibre; C, coiled duct of a sweat gland.

DISCUSSION

The observations show that in an area of acute tactile sensibility in the human skin each 'spot' is commonly innervated by two or three nerve fibres approaching from different directions, and ending in separate Meissner's corpuscles. Such an anatomical arrangement is no doubt the basis for the great tactile acuity possessed by the fingers, for it clearly provides for a spatial summation which allows of the recognition of graded stimuli. It would also seem reasonable to suppose that such an anatomical arrangement may be the

basis for tactile localization. For accurate tactile localization implies the recognition of a spatial relationship in the skin, and it may be argued that such a relationship can only be subserved by at least two impulses transmitted by separate fibres from the cutaneous area stimulated.

In the course of regeneration, nerve fibres will arrive at each separate spot at different times, because they approach it from different directions and the ultimate course followed by the individual fibres will necessarily be of different lengths. Thus in the course of regeneration there will be a phase in which each sensory spot is innervated by a single fibre instead of multiple fibres. During this phase there will be no anatomical basis for spatial summation; therefore reactions to stimuli will tend to conform to an 'all or nothing' law, leading to the characteristic explosive type of sensation, and there can be no recognition of gradation and no possibility of accurate localization. Boring suggests that if each sensory spot is projected upon the central nervous system as multiple excitations which depend for their intensity upon their relative strengths, their separation in the region of projection, and a limitation of the available amount of central energy, then multiple innervation may be effective either in summation or inhibition of the excitations involved. If this is so, then the recovery of a proportion only of the fibres innervating a sensory spot may give rise to the diffuseness and irradiation of sensation which is experienced. The same thing is seen in so-called 'intermediate zones', those areas of diminished sensibility which are found in the zones of overlap between peripheral sensory nerves after section or anaesthetization. In these areas there is an alteration in sensibility which tends to have an irradiating quality; localization is extremely poor and so are two-point discrimination and the recognition of figure patterns traced on the skin. In fact in such areas sensibility is 'protopathic' in quality. The amount of 'wicked pain' (to use Boring's apt expression) which is experienced varies in different individuals both during regeneration and in 'intermediate zones'. It is not improbable that some other peripheral factors are also involved in the production of 'wicked pain', perhaps of a chemical nature.

Many descriptions of Meissner's corpuscles have been given previously, among the most complete being that of Perez (1931), who used silver methods. He gives a description of the neural elements of the corpuscles as determined from a study of thin sections, which corresponds closely with the full pattern illustrated in Fig. 3: he also shows accessory fibres accompanying the main fibres. Perez estimated that there are 25·7 Meissner's corpuscles per sq. mm. in the adult finger pulp, and this corresponds with the present observations of between 20 and 30. His descriptions of the cutaneous nerve plexus and superficial nerve nets are also similar to those here described, but his technique, which involved the examination of thin sections of small pieces of skin, did not make it possible to trace the fibres in continuity for any distance. No mention is made of the grouping of the corpuscles, and this again would be difficult to determine by reference to thin sections.

I should like to thank Prof. H. J. Seddon for the very complete facilities which he has placed at my disposal and for permission to publish notes of a case under his care. I should also like to thank Major D. Denny Brown, R.A.M.C., for his kind co-operation and Prof. Le Gros Clark for the interest he has shown in this work and for the help he has so generously given.

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THE MOTOR CORTEX IN *GALAGO* AND *PERODICTICUS*

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Active interest in the question of the localization of the motor cortex in the Primate sub-order Lemuroidea seems to have ceased in 1908 with the publication of Brodmann's paper on cerebral cytoarchitecture in *Lemur macaco*, *Perodicticus potto*, *Propithecus coronatus*, and *Nycticebus tardigradus*. In the same year also appeared Mott, Halliburton and Kelley's studies of the excitability and cytoarchitecture of the brain in three species of the genus *Lemur*. Earlier on, May and Elliot Smith (1904) had stimulated the cortex in *Loris gracilis* and *Lemur macaco*, but no details of this investigation, or of their subsequent studies of other lemurs (Wilson & Elliot Smith, 1908), have ever been published. Apart from its general phylogenetic interest, the problem is of special interest in so far as no lemurs other than *Perodicticus* and some of the Indrisinae have an apparent central sulcus. Other members of the sub-order either have a smooth cortex where a sulcus would be expected, or at most one or two small dimples or puckers, which may be regarded as representative of the sulcus ansatus (Kappers, Huber & Crosby, 1936).

Using a unipolar method of stimulation Elliot Smith and his colleagues found that when present this "sulcus ansatus" is the caudal limit of the excitable area, an observation which agreed closely with Gordon Holmes and May's later report (1909) of the distribution of cortical degeneration which follows transection of the crus cerebri in *Lemur*. Mott and his collaborators, however, obtained a contrary result in so far as motor responses (to bipolar stimulation) were obtained not only rostral but also some distance caudal to this small sulcus, a functional finding which agreed with their later observation of large Betz cells in both regions (Mott & Kelley, 1908).

The divergence between the views of Elliot Smith and Mott is paralleled by a similar divergence in those of Ziehen (1896) and Volsch (1906) on the one hand, and Brodmann (1906, 1908), and to some extent Vogt & Vogt (1907), on the other. The rudimentary sulcus ansatus was regarded on topographical grounds by Ziehen as representing a central sulcus proper, and this view may be regarded as supported by the results of a single, and subsequently much criticized, excitation experiment carried out by Volsch, who obtained no motor responses caudal to it. Cytoarchitectural studies, however, led Brodmann to the opposite view, for he found that area 4 extended not only rostral but to a slight extent caudal to the sulcus in question. His histological observations

were corroborated by the stimulation experiments carried out by the Vogts who (as is clear from their figures) showed that the excitable area sometimes extended slightly caudal to the pseudo-central sulcus.

On the other hand both Brodmann and the Vogts found that *Perodicticus potto* has a true central sulcus which bounds the area giganto-pyramidalis caudally, and caudal to which motor responses cannot be elicited.

In putting together these various findings Kappers and his colleagues (1936) suggest in their recent review that when the sulcus ansatus is not independently defined in lemurs, it is incorporated either with the lateral, or more commonly, with the coronal sulcus. The latter, however, they state is usually incorporated in the sulcus rectus, the caudal part of which may thus represent the sulcus ansatus. It is also suggested by these authors that the sulci coronalis and ansatus combine in the Indrisinae, and presumably too in *Perodicticus*, to form a true central sulcus. Since the dorso-caudal part of the area giganto-pyramidalis in lemurs may either include the sulcus ansatus (when present) within itself, or like a central sulcus be bounded by it, it also follows that either the sulcus cannot be regarded as a definite homologue of the human central sulcus, or that the criteria by which a central sulcus has been defined in earlier studies have been drawn too finely.

The present paper provides information about a lemur, *Galago demidovii*, whose motor cortex has not been studied before, and fresh data about the motor cortex of *Perodicticus potto*.

MATERIAL

The experiments were performed on a mature male Galago, and on two female Pottos, one fully mature and the other almost mature (as judged by dentition). All operations were made with full aseptic precautions, under either a combination of sodium amytal and ether anaesthesia or ether anaesthesia alone. The limits of the excitable cortex were mapped by means of monopolar faradic stimulation, and pieces of cortex were removed by means of an electrocautery.

Cortical tissue removed at operation or autopsy was stained with methylene blue or cresyl violet.

EXPERIMENTAL RESULTS

Galago (mature male, body weight 187 g.)

Operation 1 (5 July 1933).

A large bone-flap was turned down to expose the greater part of the left cerebral hemisphere, on which the Sylvian fissure and sulcus rectus were readily defined. There was no trace of a sulcus ansatus which occasionally is represented in *Galago* by a shallow depression above the sulcus rectus; the latter, however, may sometimes be absent (Elliot Smith, 1902).

Stimulation. The cortex proved to be very unresponsive, presumably because of anaesthetic depression of the brain. The only movements elicited were

extension of the upper limb and flexion of the hip, at points between the caudal limit of the sulcus rectus and Sylvian fissure.

Extirpation. A block of cortex, including on the lateral surface the caudal part of the sulcus rectus and the dorsal part of the Sylvian fissure, and extending on the medial surface almost to the corpus callosum, was removed after occlusion of vessels with the electrocautery, serially sectioned, and stained with methylene blue.

The wound, which was closed in the usual way, healed perfectly.

Post-operative condition. The animal was very active and appeared remarkably well on the day after the operation, eating normally and showing no sign of fatigue or physical discomfort. It continuously circled to the left side and there appeared to be a definite right homonymous hemianopsia. During the ensuing nine days signs of the latter defect disappeared, but the left circling movement continued for about another week.

A well-developed right hemiplegia was present. When moving normally both upper and lower right limbs tended to slip outwards from the body, the lower limb being more affected and weaker than the upper. As a result the animal tended to fall to the right. The right hand was dragged as the animal moved, and the tail was curved to the left side and was used much less vigorously than before the operation. There was no facial or neck weakness, and the tongue was protruded symmetrically. There was a suggestion of forced grasping in the right hand, the fingers of which were clenched tightly as the animal fed. Plucking of the heel resulted in a pronounced extensor flaring of all toes.

Signs of weakness became fewer when the animal was angry or excited. Thus 24 hr. after the operation it leapt 18 in. across its cage, using its right hand and foot to grasp the bars. At no time was there any inability to move the hip, knee and ankle.

Two days after the operation the right limbs were used more actively for climbing and moving around but still only slightly for voluntary grasping, a defect from which the animal recovered a day later. The hindlimb continued to be weaker than the forelimb. Hopping and placing reactions were absent, and the animal usually failed to correct the right hindlimb when it slipped off the edge of a table. Nine days after the operation the animal had recovered further and could lift its body evenly on all four limbs, even though the right arm and leg were kept abducted. Its right foot continued to be dragged, as was occasionally the right hand, but the tail had ceased to be held asymmetrically.

Operation 2 (27 July 1933).

With a view to studying Marchi degeneration the animal was autopsied on the twenty-second day after the operation. Before an overdose of ether was given it was lightly anaesthetized, the whole cortex exposed, and an attempt made to map the motor areas of the right hemisphere.

Stimulation. The cortex was much more excitable than at the first operation, and the hind- and forelimb areas were successfully mapped (Fig. 1).

The hindlimb area was dorsal and caudal to that of the forelimb and extended about 1·5 mm. down the medial surface of the hemisphere. The area was about 4 mm. in length, with its caudal limit just behind the caudal end of the sulcus rectus.

The forelimb zone was bounded below by the sulcus rectus, below which no responses were obtained. Tail movements were associated with movements of the foot.

Representation of the digits, though rich, was not individual, and movements of a single toe or finger could not be elicited. Digital movements were always associated with movements of the knee, hip, or elbow.

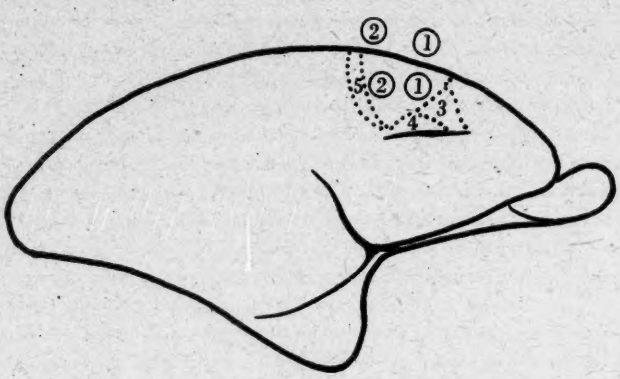

Fig. 1. Fore- and hindlimb areas on cortex of *Galago*. 1, Flexion of knee and hip. 2, Flexion of knee and hip, toes extended, tail moved laterally. Movements of the tail were most pronounced in the caudal part of the excitable hindlimb area (5). 3, Raising of shoulder, flexion of elbow, some flexion of fingers. 4, Flexion of fingers and elbow. The excitable area extended 1·5 mm. on to the medial surface of the hemisphere.

Histology. The piece of left cortex removed at the first operation and pieces from all but the caudal third of the right hemisphere were serially sectioned.

The excitable areas of the cortex were differentiated from surrounding zones, but not as distinctively as in the ape or human brain. Thus some cells of the inner granular zone could still be recognized between the otherwise merged layers 3 and 5, the histological picture being somewhat similar to that in *Tarsius* (Woollard, 1925). Furthermore, the Betz cells, while apparently larger than in *Tarsius*, were not as large relatively as in man.

In spite of this somewhat indistinct picture of a motor cortex, the area above and below the sulcus rectus and extending back to midway between the caudal end of the sulcus rectus and the upper end of the Sylvian fissure could be distinguished from surrounding zones in which the inner granular layer was better marked, even though in these non-motor zones the granular layer was partly merged with layers 3 and 5.

Potto I (adolescent female, body weight 600 g.)

Operation 1 (10 July 1933).

A large bone-flap was turned down, under ether anaesthesia, to expose most of the left cerebral hemisphere, the chief markings of which were traced on cellophane.

Stimulation. The cortex was relatively unresponsive, presumably because anaesthesia was too deep. Discrete movements of the fingers were obtained on stimulation of an area about 4 mm. square lying between the central sulcus and the caudal end of the sulcus rectus.

No movements of the lower limb were obtained by excitation of the lateral surface of the hemisphere. Primary movements of the hallux and other toes were obtained, however, on stimulation of the medial surface about 1 mm. from the upper border, and in front of a line opposite the central sulcus.

Extirpation. A block of cortex, including the central sulcus and sulcus rectus, was removed. The animal, however, died under the anaesthetic, and no further observations were made.

Histology. See below.

Potto II (mature female body weight 850 g.)

Operation 1 (26 July 1933).

The left cerebral hemisphere was exposed under amytal anaesthesia in the usual way, and the course of the central sulcus and sulcus rectus traced on cellophane to provide a map of excitable points.

Stimulation. The cortex was very excitable.

No motor responses were obtained from any point behind the central sulcus, and none below the level of the sulcus rectus and a line joining it to the central.

The excitable area extended from above this line, over the superior border of the hemisphere and 3 mm. down the medial surface. Behind it was bounded by the central sulcus and a line projected upwards from it; in front it passed forwards to a line projected upwards from the junction of the middle and caudal thirds of the sulcus rectus (cf. Fig. 2).

The upper limb was represented in the lower half of this area, and the lower limb in the upper, extending on to the medial surface.

The upper limb area was subdivided, so that the fingers were represented in its lowest and the shoulder in its uppermost part. Representation was reversed in the lower limb area, the hip being most ventral and the toes extending on to the medial surface. Points at which extension movements of the fingers were obtained were distinct from those which gave flexion.

There was no 'dead zone' between the lower and upper limb areas; the points at which shoulder movements were obtained were immediately ventral to points of which stimulation led to movement of the hip.

More than half of the hindlimb area was devoted to the toes, and discrete

movements of the hallux were related to one small zone. Flexion movements of the toes were not obtained at any time.

No trunk, head or neck movements were observed during this experiment. At its conclusion the cortical region concerned with the lower limb was extirpated, and the wound closed in the usual way. Before considering the motor defects that followed this operation, the results of a second experiment, when the right hemisphere was stimulated, may be conveniently described.

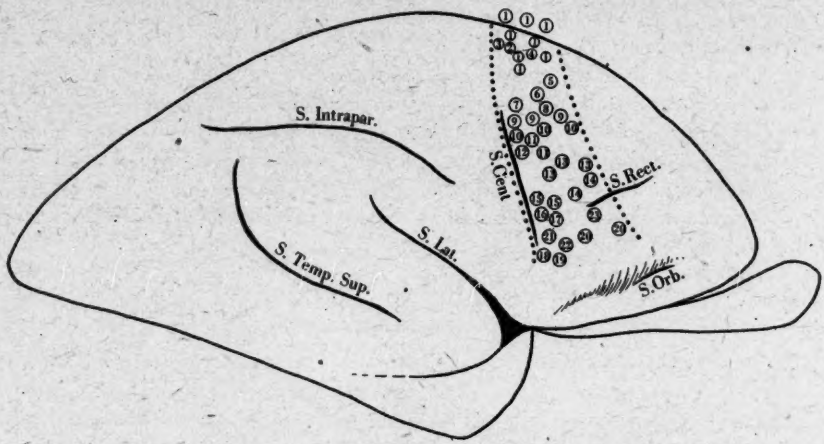

Fig. 2. Motor representation in cortex of *Perodicticus*. 1, Extension of toes. Excitable area extended 3 mm. on to medial surface of hemisphere. 2, Extension of hallux alone; 3, extension of ankle; 4, extension of ankle with slight extensor movement of proximal phalanges of toes; 5, flexion of toes, slight withdrawal of leg; 6, flexion of knee and ankle; movement, to right, of lower part of vertebral column and tail; 7, flexion of hip; 8, flexion of knee; 9, movements of shoulder; 10, adduction of arm, flexion of elbow; 11, extension of fingers, movement of pollex weakest; 12, flexion of fingers, slight flexion of elbow, adduction of arm; 13, extension of fingers, particularly pollex (cf. 11); 14, extension of pollex alone; 15, flexion of fingers; 16, extension of neck and drawing up of shoulder; 17, lifting of head; 18, lifting of head, retraction of snout; 19, movement of scalp and ears; 20, protrusion of head; 21, extension of neck; 22, nodding of head; 23, quickening of breathing movements of nostrils and diaphragm.

Operation 2 (15 August 1933).

The whole cortex was exposed, preparatory to autopsy, under light ether anaesthesia, and the sulci traced on cellophane.

Stimulation. The cortex was very responsive to faradic stimulation, and in addition to confirming and extending most of the observations made before on the left hemisphere, it was possible to map the head and neck areas on the right hemisphere. As in the first experiment, no responses were obtained caudal to the central sulcus, and on this occasion none was obtained from the medial surface of the hemisphere.

Fig. 2 is a composite diagram embodying the results of stimulation of both hemispheres (operations 1 and 2).

Apart from the general conclusions referred to above, the following points emerge:

(1) The cortical zone of representation for head and neck lies ventral to the sulcus rectus and a line projected from it to the central sulcus.

(2) The area in front of the central sulcus, which is about 5·5 mm. long, is almost entirely devoted to the upper limb. Representation of the hand is extensive, and discrete movements of the pollex can be obtained rostral and somewhat ventral to the area related to the other fingers.

(3) The trunk appears to have an insignificant representation, and its movements are closely linked with those of the hindlimb.

(4) Representation of the hip is caudal to that of the knee, while that of the hallux also seems to be more caudal than that of the other toes.

It may be noted that flexor movements were obtained mainly at the beginning of the experiment, and became more difficult to elicit the longer the cortex was kept exposed. Furthermore, the fact that the medial surface of the hemisphere was unexcitable in the second experiment, whereas it gave responses in the first, may be related to the fact that in the former it was not approached before the cortex had been exposed and stimulated for more than an hour.

Extirpation. As stated above, the left cortical zone in which the lower limb was represented was extirpated at the first operation, and the animal closely watched during the ensuing twenty days.

The morning after the operation the right lower limb appeared paralysed except for some weak hip flexion. The limb was dragged during locomotion.

Later in the day, however, the muscular weakness seemed to lessen, and movements (including grasping by the foot) were demonstrated in all the segments of the limb. The movements were, however, slower and less co-ordinated than in the opposite limb. The strength of the grasp of the two feet seemed approximately equal, although the flexor defence mechanism was quicker and much more powerful with the left leg and foot.

This picture of paresis was sustained for the three weeks that intervened between the operation and the autopsy. On the day it was autopsied it was noticed when the animal was lying on its back that it kept groping with its left foot for something to grasp, and that it made no attempt to do so with the right. The possibility that the defect in the activity of the right limb was partly due to some measure of sensory loss was tested, but not confirmed.

Histology. As indicated in the brief account given above of the histological characteristics of the motor cortex in *Galago*, our findings confirm Brodmann's view (1906, 1908) that cortical lamination in the Lemuroidea is less distinct than in monkeys and man. The differentiation of the motor cortex in *Perodicticus* is, however, clearer than in *Galago*, not only because it is deeper than adjacent zones and because it is sharply limited by the base of the central sulcus, but also because (*a*) the internal granular layer which is distinct, for example, immediately behind the sulcus, is unrecognizable as a layer in front

and (*b*) because of the conspicuousness of the giant Betz cells in layer 5 (Fig. 3).

These points were established by examination of the brain of Potto I, the left leg area and all the rostral part of the right hemisphere of Potto II, and the brain of a control Potto. Definite motor cortex was found over the whole area that proved responsive to faradic stimulation, including the upper part of the

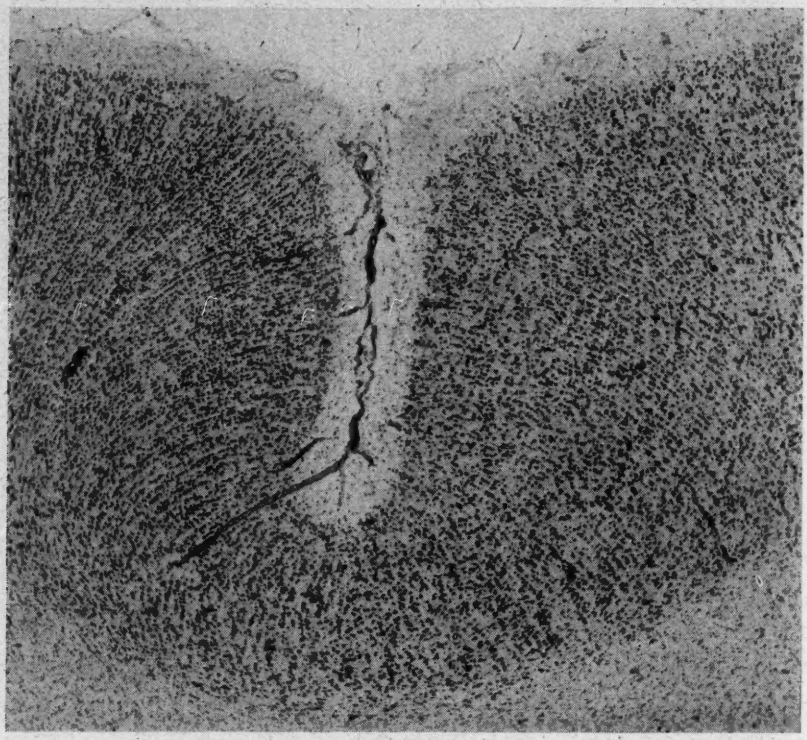

Fig. 3. Central sulcus of *Perodicticus* with motor cortex to right. ×37.

medial surface of the hemisphere opposite the lateral excitable area. Large pyramidal cells were also found in layer 5 outside the excitable area, but the non-motor regions in which they occurred were characterized by a fairly clear internal granular layer. Some diffusely scattered internal granular cells were also found in the motor area proper, but nowhere did they form a distinct layer; in general, excitable areas constituted an agranular cortex.

DISCUSSION

The observations noted above define the motor areas in the cortex of *Galago* and *Perodicticus* by stimulation and extirpation experiments as well as by histological methods. The only previous extirpation experiments on Lemuroidea are those of Mott and Halliburton who, in *Lemur catta* and *Lemur macaco*, removed variable amounts of motor cortex from four animals and noted that post-operative paralysis was only transitory. As already mentioned, our own observations confirm this conclusion so far as the acute effects of the operation are concerned, but suggest that signs of motor defect are still demonstrable three weeks after the operation.

Our observations also confirm and extend those the Vogts provide about the motor cortex in *Perodicticus*. The number of discrete representational points noted by them is fewer than in the present experiment, but includes two—loci for tongue and eye movements—which we failed to demonstrate. It is also clear that in the case of the present *Galago* experiment the lack of head and neck responses can be related to the unexcitability of the cortex ventral to the caudal end of the sulcus rectus, for, as the Vogts have shown, the head area in lemurs is generally situated in this region.

In discussing the homologies of sulci, the Vogts define three grades of resemblance. The first is when comparable sulci in two animals lie either completely within identical functional and cytoarchitectural fields or when similar fields have their boundaries in identical positions within comparable sulci. The second grade of homology occurs when two sulci resemble each other generally from the topographical point of view but where the extent to which they form limiting sulci to the same cortical field varies. The third is where sulci do not resemble each other topographically, and bear some, but not identical, relationships to the limits of the same cortical field. The Vogts carefully point out that the three stages of homology grade into each other, and that they do not represent distinct degrees of homology.

Accepting these distinctions it would seem, as the Vogts suggest, that the central sulcus in *Perodicticus* bears a second-grade homology to the human central sulcus, and that the sulcus ansatus in other lemurs represents no more than an homology of the third degree. Brodmann doubts whether the sulcus ansatus can in any sense be regarded as homologous with the central sulcus, but accepts the homology of the central sulcus in *Perodicticus* with that of higher primates. Our observations suggest that the homology is mainly with that part of the human central sulcus which bounds the upper limb area.

SUMMARY

1. The motor area in *Galago* is not bounded either rostrally or caudally by sulci. It extends below and above the sulcus rectus and on to the upper part of the medial surface, the hindlimb area being dorsal to the forelimb zone.

2. In *Perodicticus* the motor area lies rostral to the central sulcus and a line projected upwards and downwards from its dorsal and ventral extremities. The general disposition of representational parts of the body is the same as in man.

3. Extirpation of the motor area in both species leads to motor defects which rapidly clear up, but which, over a period of three weeks, are still demonstrable.

The experiments on which this paper is based were made, in 1933, when S. Zuckerman was a Rockefeller Fellow in the Laboratory of Physiology, Yale University School of Medicine. Our best thanks are due to Prof. W. E. Le Gros Clark for his help in the histological section of this work.

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THE NERVE SUPPLY OF THE MAMMALIAN DUCTUS ARTERIOSUS

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Koch (1931) was the first to suggest, on theoretical grounds, that specialized pressor-receptor nerve-endings are found on those arteries of the adult mammal which are persistent portions of the embryonic branchial arch arterial pattern. The work of Ask-Upmark (1935) and observations published by the writer (Boyd, 1934, 1937*a*) support, in a general way, Koch's suggestion, and the studies of Tello (1924), de Castro (1928), Nonidez (1935), Muratori (1937) and Tschernjachiwsky (1938) have shown that there is an ample histological basis for the conclusion that the persistent portions of the third and fourth branchial arch arteries are provided with a special and characteristic afferent innervation. This innervation forms the receptor element in the aortic and carotid sinus proprioceptive reflex arcs. The presence of such specialized pressor-receptor regions in those arteries of the adult mammal which are derived from the third and fourth arch vessels has caused several investigators to postulate the presence of equivalent receptors in the pulmonary trunk and its main branches but the evidence for such a pulmonary arterial pressor-receptor mechanism is still inconclusive.

Koch (1931) quotes the observation of v. Schumacher (1902), Perman (1924) and Tello (1924) as evidence for a specialized nerve supply to the pulmonary arteries. These investigators, however, were concerned only with the general relations of the nerves to the pulmonary vessels and none of them described nerve endings in the pulmonary artery of the type now recognized as specifically pressor-receptor in function. Takino (1933), and more recently, Takino & Watanabe (1938), as a result of extensive investigations of the nerve-endings in the pulmonary arteries of a number of mammalian types, conclude that these vessels, and especially their extra-pulmonary portions, do receive a rich supply from sensory nerves. Indeed, they state that this supply is richer than that obtained from the vaso-motor nerves. Nonidez (1935), on the other hand, has stated that there is a complete and uniform absence of pressor-receptors from the wall of the pulmonary trunk and the proximal portions of the pulmonary arteries. 'This point', he writes, 'can be established beyond reasonable doubt through the study of well impregnated sections of the hearts of new-born animals. When the size and characteristics of the pressor-receptors are considered it seems highly improbable that they would escape notice or that they failed to be impregnated.'

The physiological evidence is also inconclusive. Schwiegk (1935) has described slowing of the heart and a fall in the systemic blood pressure in the

dog when the pressure within a perfused pulmonary artery is raised. Schweitzer (1936), who made similar experiments in the cat, obtained mainly negative results. He writes: 'in the cat, pressure changes in the pulmonary circulation are only rarely associated with changes of systemic arterial pressure and heart rate. This is in marked contrast to the regularity of response obtained from the carotid sinus.' To explain this difference Schweitzer suggested that destruction of the afferent nerves in the adventitia of the pulmonary artery during insertion of the cannula was responsible for the negative findings. In view of the difficulty presented by denervation of the carotid sinus or aorta, however, this explanation does not seem easily acceptable.

Takino & Watanabe (1938) have also described slowing of the heart and a marked fall in blood pressure following mechanical or electrical stimulation of the pulmonary artery in the region of attachment of the ligamentum arteriosum of adult rabbits. The bradycardia and fall in blood pressure were not obtained when the stimulation had been preceded by sectioning of the left aortic (depressor) nerve and the left vagus. As these workers also identified pressor-receptor nerve-endings in the walls of the pulmonary artery their contribution lends considerable support to those who hold the opinion that the pulmonary arteries possess a nervous mechanism similar to that found in the carotid sinus and the aorta.

In addition to portions of the pulmonary arteries the ductus arteriosus is also generally regarded as being derived from the sixth branchial arch arteries of the embryo. Consequently a number of investigators (Watanabe, 1938; Muratori, 1937; Boyd, 1937*b*; Takino & Watanabe, 1938; and Tschernjachiwsky, 1938) have examined the nerve supply of the ductus arteriosus, and the results obtained by all these workers indicate that there is a sufficient histological basis for the assumption that, at any rate in foetal life, the ductus possesses a pressor-receptor apparatus similar to that found in the carotid sinus, the aortic arch and the innominate-subclavian arch of the right side.

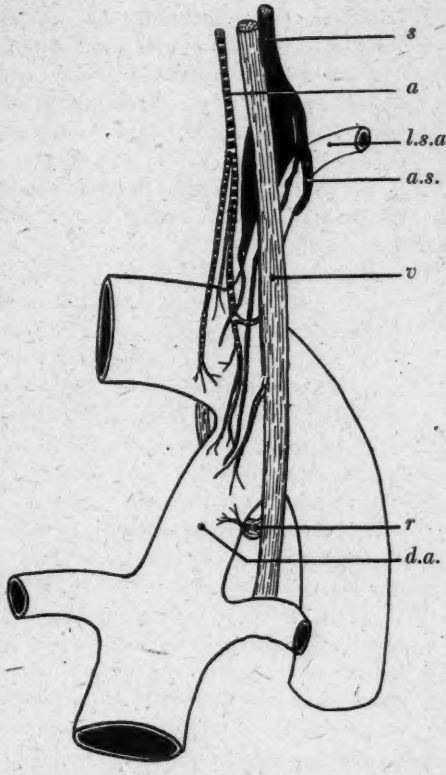
Since publishing a note (1937*b*) indicating that the ductus arteriosus of the rabbit possesses a specialized innervation I have collected material bearing on the subject from a number of mammalian species (pig, cat, dog, rat, mouse and man), and the purpose of this paper is to describe the results of a study of this material. As the material includes in many cases portions of the pulmonary arteries, some comment will be made on the nerve-endings found in these vessels, but the main object in view is a description of the nerve supply to the mammalian ductus arteriosus.

MATERIAL AND METHODS

The available material included transverse, sagittal and coronal serial sections through the thorax and root of the neck of rabbit embryos and foetuses of various developmental stages up to, and including, new-born specimens. A number of series of sections through the ligamentum arteriosum and adjacent portions of the aorta and main pulmonary arteries of adult

rabbits have also been used. Some of this adult material was derived from rabbits on whom the operations described in an earlier publication (Boyd & McCullagh, 1938) had been performed, and included specimens from animals in which either the left vagus nerve, or the left aortic ('depressor') nerve, or both, had been sectioned a considerable time before the death of the animal. In addition to this rabbit material, sections are available through the ductus arteriosus of various developmental stages of pig, mouse and human embryos and foetuses, and of new born and adult specimens of these mammals and of cat, dog and rat.

Most of the sections are from series stained *en bloc* with one or other of the Cajal techniques, the younger embryos with the pyridine method, the older embryos and full-time foetuses with the de Castro (1925) modification. A few series were stained by the Bielschowsky or Bodian techniques.



Text-fig. 1. Schematic reconstruction of nerve supply to the ductus arteriosus in rabbit. *s*, Sympathetic trunk; *a*, left aortic nerve; *l.s.a.* left subclavian artery; *a.s.* ansa subclavia; *v*, left vagus nerve; *r*, recurrent nerve; *d.a.* ductus arteriosus.

OBSERVATIONS

Most of the available material was obtained from the rabbit and, consequently, this mammal will be used as the basis for the description. The variations found in the nerve supply to the ductus arteriosus in the other mammals will be referred to at the end of this description.

Text-fig. 1, which is a schematic reconstruction of the region of the ductus arteriosus in a rabbit foetus nearly at full term gestation, shows the relationships between the ductus arteriosus and the nerves found in all the specimens from the 22 mm. stage of development. In all the embryonic and foetal rabbit material branches of the left aortic ('depressor') nerve can be traced beyond the aortic arch to the ductus. Some of these fibres reach the ductus along the wall of the aorta so that the appearance is as if the aortic pressor-receptor area had spread on to the attached ductus arteriosus. Other fibres, however, and for the most part from the division of the left aortic nerve which is

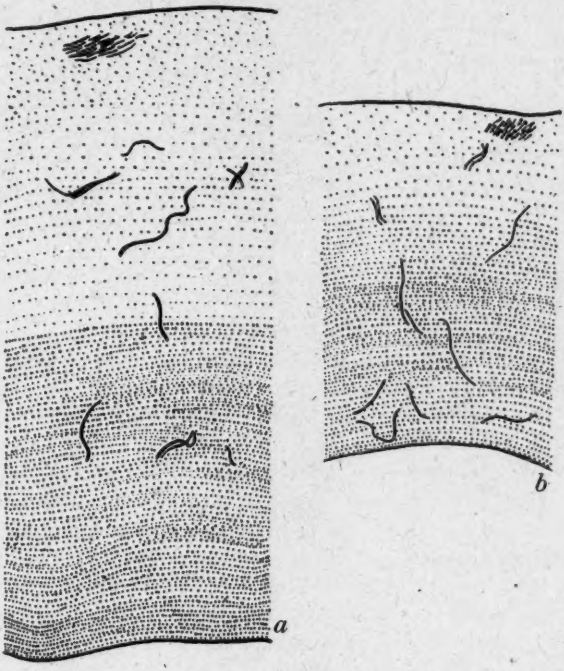
supplying the dorsal aspect of the aorta, leave the aorta and pass away from its wall to reach the ductus at some distance from the point at which it enters the former vessel (Pl. 1, fig. 2). The aortic nerve itself usually bifurcates a short distance above the aortic arch and, at the point of bifurcation, is related to the collection of cells similar to those of the carotid body, which Nonidez (1935) has called the aortic glomus. The bifurcation results usually in a larger anterior and a smaller posterior division which are distributed to the corresponding surfaces of the aortic arch in a limited area to the right of the origin of the left subclavian artery but not quite reaching the commencement of the brachiocephalic trunk. In most of the embryonic and foetal material the supply from the aortic nerve to the ductus arteriosus is distinctly greater from the posterior division, but in several embryos the anterior division appears to be the sole contributor. In many specimens it is possible to trace fibres from the sympathetic cord to the depressor nerve in the lower part of its course, or to one or both of its divisions. Some of these sympathetic fibres appear to be terminating in relation to the cells of the aortic glomus; others seem to continue in the aortic nerve bundle to the wall of the aorta, but the material does not permit of comment on their ultimate distribution.

In addition to this supply from the left aortic nerve the ductus arteriosus receives a supply from the left vagus trunk as it crosses the vessel (P. 1, fig. 1). Once the fibres have entered the adventitia of the ductus it is impossible to separate those of aortic nerve origin from those of vagal origin, but my impression is that the vagus contributes chiefly to the antero-inferior aspect of the vessel while the aortic nerve is concerned mainly with the innervation of its postero-superior surface. In a few specimens the inferior aspect of the ductus arteriosus receives some fibres from the recurrent laryngeal nerve as this nerve hooks under it (Pl. 1, fig. 3). These fibres may be presumed to be vagal fibres which are leaving the nerve at a lower level than is usual and which accompany the recurrent nerve for a short distance. Finally, some fibres appear to reach the ductus arteriosus from the lower cervical and upper thoracic sympathetic cord of the left side. Certainly fibres of sympathetic origin are closely related to the terminal ramifications of the aortic nerve and, in some specimens, it has been possible to trace distinct bundles from the sympathetic trunk into the vagus nerve and, more rarely, to the ductus itself. In serial sections, however, it is impossible to be definite, and as the experimental results on adult rabbits made no contribution to this aspect of the problem, the relationship of the sympathetic fibres to the ductus is left undecided by the material available.

On reaching the ductus arteriosus, the fibres from the aortic nerve and the vagus trunk course for a variable distance in the adventitia and eventually terminate in endings which are very similar to those found in the carotid sinus and the aortic arch. In the younger embryos (see Text-fig. 2) the fibres tend to penetrate further into the wall of the ductus, approaching, and sometimes reaching, the intima. The fibres which reach the intima usually bend back

into the muscular coat of the ductus and they represent the 'fibres égarés' described by Tschernjachiwsky (1929) in the carotid sinus of the human embryo. In the older embryos and new-born foetuses (see Text-fig. 2) the fibres are for the most part restricted to the outer third of the media and to the adventitia, but a few fibres can always be traced more deeply into the wall of the ductus.

The fibres which pass into the wall of the ductus are for the most part thick and possess a well-marked medullary sheath. These fibres terminate in endings



Text-fig. 2. Camera lucida drawings of nerve fibres in the wall of the ductus arteriosus of the rabbit. *a*, Full term foetus; *b*, 32 mm. embryo.

(Text-fig. 3; Pl. 1, fig. 4) which are markedly branching and which possess small thickenings and rings on the branches very similar to those found on the nerve terminations of the other pressor-receptor areas. The endings in the ductus, however, are not as extensively ramified as are the better known ones and occasionally are very circumscribed. Endings of these thick fibres other than of the branching variety have not been found and none of the endings are encapsulated.

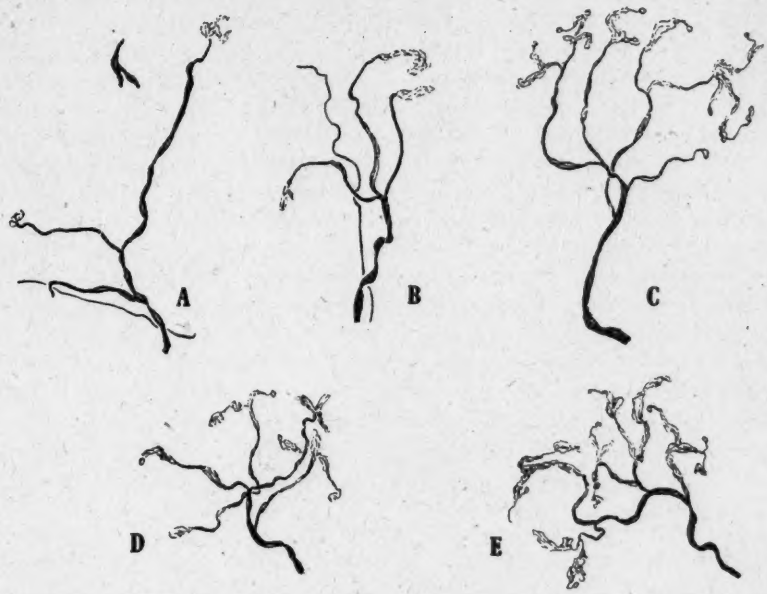
In addition to these thick fibres, and their endings, which constitute the major nerve supply to the ductus arteriosus, other and much finer fibres can

be found to enter it (Pl. 2, fig. 1). These fibres are very finely myelinated, and they terminate in relation to the muscle fibres of the wall of the ductus by simple endings which resemble the endings of vaso-motor fibres in other blood-vessels. They appear, then, to be motor fibres. They may be of sympathetic origin, but some of them could be traced directly from the left vagus nerve to the ductus. This fact, however, does not preclude their ultimate origin from the sympathetic trunk. As has already been stated, my material is not suitable for determining the real nature of the relationship of the sympathetic nervous system to the ductus arteriosus. Nonidez (1939) has recently described a marked difference in the capacity for silver reduction of sympathetic and parasympathetic post-ganglionic fibres in material stained by the Cajal technique, the former fibres staining much more faintly. Unfortunately my material does not show this differentiation clearly enough to allow a decision to be made on this basis alone although it does give grounds for support of Nonidez's contention.

After birth the ductus closes and eventually is represented by the tough fibrous band which is called the ligamentum arteriosum. In the earlier stages of this investigation this embryonic vestige was ignored, but the work of Takino & Watanabe (1938) directed attention to it and its nerve supply has now been studied in six normal adult specimens. In all of these specimens the statement of these workers that the ligamentum arteriosum retains part of the nerve supply which it possesses in foetal life could be verified, but my material shows fewer endings in the ligamentum than they appear, from their account, to have found. In the adult rabbit material examined in this investigation the sensory endings are sparse in comparison with the embryonic and foetal ductus but they are unmistakeable and, like the foetal endings, show a close resemblance to those found in the other arterial pressor-receptor areas (see Pl. 2, fig. 7). As sections of the aorta and adjacent vessels from the rabbits which had been used for investigations on the results of chronic denervation of the pressor-receptor areas (Boyd & McCullagh, 1938) are available, it has been possible to study the changes in the nerve-endings in animals in which the left vagus and left aortic nerves had been sectioned. In rabbits with both of these nerves cut, endings are completely absent from the ligamentum arteriosum. This finding corroborates the description which has been given for the embryonic and foetal ductus and demonstrates that this portion of the sixth branchial arch artery receives a nerve supply from both the left aortic nerve and the left vagus trunk. Further, in those rabbits in which only the left vagus or left aortic nerve had been sectioned endings could always be found (eight specimens) in the ligamentum arteriosum. This finding again supports the conclusion arrived at from a study of the embryonic and foetal material that there is a double origin of the fibres supplying the afferent innervation to the ductus. The material, however, does not permit of a statement on the relative contributions from these two sources to the ductus. In rabbits in which the right aortic and right vagus nerves had been sectioned no

change was found in the sensory endings of ths ligamentum arteriosum. This is in agreement with results obtained by Takino & Watanabe and strongly suggests that the nerve supply to the ductus is derived solely from the nerves of the left side. In none of the adult material have the finer fibres been found and it is, therefore, not possible to comment on the results of vagal section on them.

The findings reported for the ductus arteriosus of the rabbit differ in no essential manner from those obtained in the other mammalian species which have been investigated. Examples of the sensory endings found in the different



Text-fig. 3. Sensory nerve-endings in the wall of the ductus arteriosus of A and B, rabbit; C, cat; D, dog; E, man.

species are shown in Text-fig. 3. The rabbit is a special case in that it possesses constantly a separate aortic ('depressor') nerve and, with the exception of the rat where the nervous relationships are very similar to those found in the rabbit, the sensory nerve supply of the ductus in all the other species investigated appears to be derived from the vagus nerve alone. The pig does possess a separate aortic nerve on the left side but this is distributed solely to the aortic arch and could not be found to reach the ductus arteriosus. Watanabe (1938) reports that he was unable to find sensory endings in the ductus of young pigs. My embryonic pig material (18–100 mm. stages) shows a rich innervation of the ductus, but in the ductus of the only new-born pig

investigated by me I was unable to find sensory endings. Whether this was due to faulty technique in staining or to a real absence of the endings in the new-born pig I am unable to state.

In none of the material from any of the mammals which have been studied has a rich afferent nerve supply been found in the walls of the distal part of the pulmonary arterial trunk or in its right and left branches. This is particularly true for the embryonic material where the absence of nerve fibres from the walls of these vessels contrasts markedly with the rich supply to the pressor-receptor areas of the aortic arch and the ductus arteriosus. Nevertheless, occasional ramified nerve-endings are to be found in the pulmonary vessels (Pl. 2, fig. 8). These endings, however, are never so richly branching as are those found in the other regions and they are much more scattered. It has not been found possible to identify in any of the material the area of spread of nerve-endings from the ductus to the adjacent part of the pulmonary trunk as has been described by Takino & Watanabe in their rabbit sections. On the contrary, my material shows a marked diminution in the number of endings in the ductus arteriosus as one passes from the aorta to the pulmonary artery, and the pulmonary extremity of the ductus appears completely free from sensory endings. Further investigation with more adequate material has convinced me, however, that my earlier denial (1938) of a specialized afferent nerve supply to the pulmonary trunk was mistaken. But the supply of sensory endings to the pulmonary vessels in the portions examined is so sparse and their character so simple, in comparison with those in the aorta and ductus arteriosus, that it seems justifiable to conclude that the physiological significance of the former will be found to differ both quantitatively and qualitatively from that of the better known pressor-receptor areas.

DISCUSSION

Nonidez (1935), in his description of the aortic nerve and the glomus aorticum in the rabbit, briefly referred to a few nervé fibres terminating in the wall of the ductus arteriosus 'by means of arborizations of a much simpler type than those found in the aorta'. He stated that in no specimens were any fibres seen entering the pulmonary trunk itself and he suggested that the endings in the ductus might play a role during foetal life. My preparations, and the work of Takino & Watanabe, completely verify Nonidez's description but show that the nerve supply is richer than he suspected. The recent work of Barcroft and his collaborators on the foetal circulation would seem to give a special significance to these histological findings. Their work has shown quite clearly (Barcroft, 1938; Barcroft *et al.* 1939) that the changes in the ductus arteriosus at birth are abrupt and that *functionally* there is a rapid obliteration of its lumen. Anatomical obliteration of the lumen is not a sudden process and may take several weeks or months for its completion, so that 'probe patency' of the ductus will be present for some time after blood has ceased to flow through it. When a histological basis for the sudden closure of

the ductus is looked for it can be found in the structure of its wall which has, when compared with the pulmonary trunk and the aorta, an increase in the amount of muscular tissue and a marked diminution in the number of elastic and collagenous fibres.

Through the kindness of Prof. Barcroft I have been able to examine the structure of the aorta, the pulmonary artery and the ductus arteriosus in a number of sheep foetuses before, at, and after full-term gestation. In sections stained with van Gieson the wall of the ductus in the sheep foetus takes up practically none of the acid fuchsin, resembling closely in tinge the staining of the heart musculature. On the other hand, the walls of the aorta and pulmonary arteries have a markedly reddish tinge. With slight differences in detail the results obtained on the ductus arteriosus of other mammals (dog, cat, rabbit, man) are similar, and in material stained for elastic tissue the ductus shows almost complete absence of elastic fibres in the media, a result once again in marked contrast to the richness of the aorta and the pulmonary vessels in these fibres. The ductus arteriosus, then, is almost exclusively a 'muscular' artery while the pulmonary artery and the aorta are 'elastic' vessels, and it seems most probable that this difference in structure of the ductus from that of the adjacent segments of the pulmonary arteries and the aorta is associated with the peculiar behaviour of the ductus at birth.

The mechanism causing the contraction of the ductus arteriosus at birth and responsible for the maintenance of the contraction during the period necessary for anatomical obliteration of its lumen is not yet adequately understood. An explanation of the part played by the musculature of the wall of the vessel in the process has been given by Hayek (1935) who showed, by a geometrical analysis, that the peculiar obliquity of the muscle fibres in the wall of the human ductus arteriosus can greatly facilitate the physiological obliteration of its lumen at birth. His explanation seems acceptable as a statement of the functional significance of the musculature of the ductus in the mechanism of closure but it does not include an explanation of the factors involved in the control of the muscular contraction. Any explanation of the mechanism of closure of the ductus arteriosus at birth will have to take into consideration the nerve supply to this arterial segment, for physiological closure of the ductus must be the result of special muscular and nervous conditions obtaining in the wall of the vessel.

The finer fibres which have been described in the media of the foetal ductus are presumably the efferent fibres to its musculature and are probably concerned in the initiation and maintenance of the contraction of the sphincter-like muscle. Unfortunately the inherent difficulties of histological investigation on serial sections do not permit of a definite statement on the origin of these fibres. They appear, in foetal material, to be coming from the left vagus nerve, or its branches, but the connexions, at a higher level, between the vagus and the sympathetic trunk make it impossible to exclude a contribution from the latter source, and, indeed, some of the fibres appear to come to the ductus from

the sympathetic trunk directly. As the finer fibres have not been found in the adult ligamentum arteriosum, the sections from those rabbits in which the left vagus and aortic nerves had been cut can throw no light on the problem. Some results obtained by Barcroft and his co-workers (1938) in the guinea-pig foetus are suggestive, for they showed that stimulation of the left vagus nerve produced blanching of the ductus, presumably due to contraction of its musculature, but the possibility of sympathetic fibres being involved in the stimulation still holds for this experiment. These workers showed further, however, that the addition of pilocarpine to the Ringer's solution being used to perfuse the ductus produced some degree of constriction of the vessel and in two out of three experiments atropine opened the ductus after the pilocarpine constriction. The experiments are not conclusive, and the investigators stressed their applicability only to the guinea-pig, but the results suggest a parasympathetic rather than a sympathetic supply to the musculature of the ductus.

It is difficult to assess the functional significance of the pressor-receptors found in the adventitia and outer portions of the media of the ductus arteriosus. The endings will be stimulated, mechanically, by changes in the pressure within the ductus or by contraction of the muscular coat of the ductus. During the later part of foetal life, therefore, they may be concerned in reflexes which aid in the control of the foetal blood pressure, though the evidence for a control at this stage of development is poor. At birth, when the circulation of blood through the ductus ceases, they may be concerned in initiating reflexes which maintain the contraction of the musculature of the ductus until anatomical obliteration of the lumen is complete. After complete closure of the ductus and atrophy of its musculature some of the sensory endings may retrogress, but my material and the results obtained by Watanabe & Takino show that a number of the endings persist into adult life. Nonidez did not explicitly state that he found such endings in the ligamentum arteriosum of the adult rabbit but his statements suggest that he did for he writes 'the possible function of nerve-endings in the wall of the obliterated duct (ligamentum arteriosum of the adult) can only be surmised'. Whatever the explanation may be it is quite definite that some, at least, of the sensory endings concerned in the supply of the foetal ductus do not retrogress when anatomical obliteration of its lumen occurs.

Finally it must be stressed that the sensory nerve supply to the ductus arteriosus (and possibly the sparser supply to the pulmonary trunk) fits in very well with the explanation suggested by Koch for the distribution of the major arterial pressor-receptor areas. These areas are found on the portions of the branchial arch arteries which persist into adult life. Pressor-receptor endings of various sorts are found elsewhere in the vascular system, notably in the heart wall and in the great veins entering the right atrium (Nonidez, 1937). The wide distribution of such pressor-receptors, however, does not make less significant the striking fact of the concentration of specialized receptors

in localized parts of the vascular system where they have apparently a special physiological significance. That one of the regions with this special significance is constituted by the branchial arch arteries of lower vertebrates is not surprising in view of the position of these vessels in relation to the outflow from the heart and the supply to the thin-walled vessels of the gills. In higher vertebrates the mechanism has been retained on the equivalent vessels and, whatever the ultimate explanation of this retention may be, the presence of an apparatus for recording the intravascular pressure on the major arterial outflows from the heart has an obvious importance in the reflex control of the circulation.

SUMMARY

The mammalian ductus arteriosus possesses a sensory innervation very similar to that possessed by the aorta and the carotid sinus. This nerve supply is derived from the left vagus nerve, and, when it is present, from the left aortic nerve. Fine nerve fibres, presumably motor, are also found terminating in relation to the muscular coat of the ductus, but the available material does not permit of a statement as to their origin. The distal portion of the pulmonary trunk and the proximal portions of the pulmonary arteries also possess a sparse afferent innervation.

The nerve supply of the ductus arteriosus is discussed in relation to the behaviour of the ductus at birth.

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EXPLANATION OF PLATES 1 AND 2

Plate 1

Fig. 1. Coronal section of the ductus arteriosus of a full-term rabbit foetus. The section is viewed from behind. Branches of the left vagus nerve can be seen in the wall of the ductus. de Castro technique. ×60.

Fig. 2. Transverse section of a portion of the wall of the ductus arteriosus of a full-term rabbit foetus. Fibres from the left aortic nerve are entering its wall. de Castro technique. ×60.

Fig. 3. Coronal section of the ductus arteriosus of a full-term rabbit foetus. The section is viewed from behind and fibres from the left recurrent nerve can be seen entering it. de Castro technique. ×60.

Fig. 4. Section of the wall of the ductus arteriosus of a full-term rabbit foetus to show pressor-receptor nerve-endings. de Castro technique. ×390.

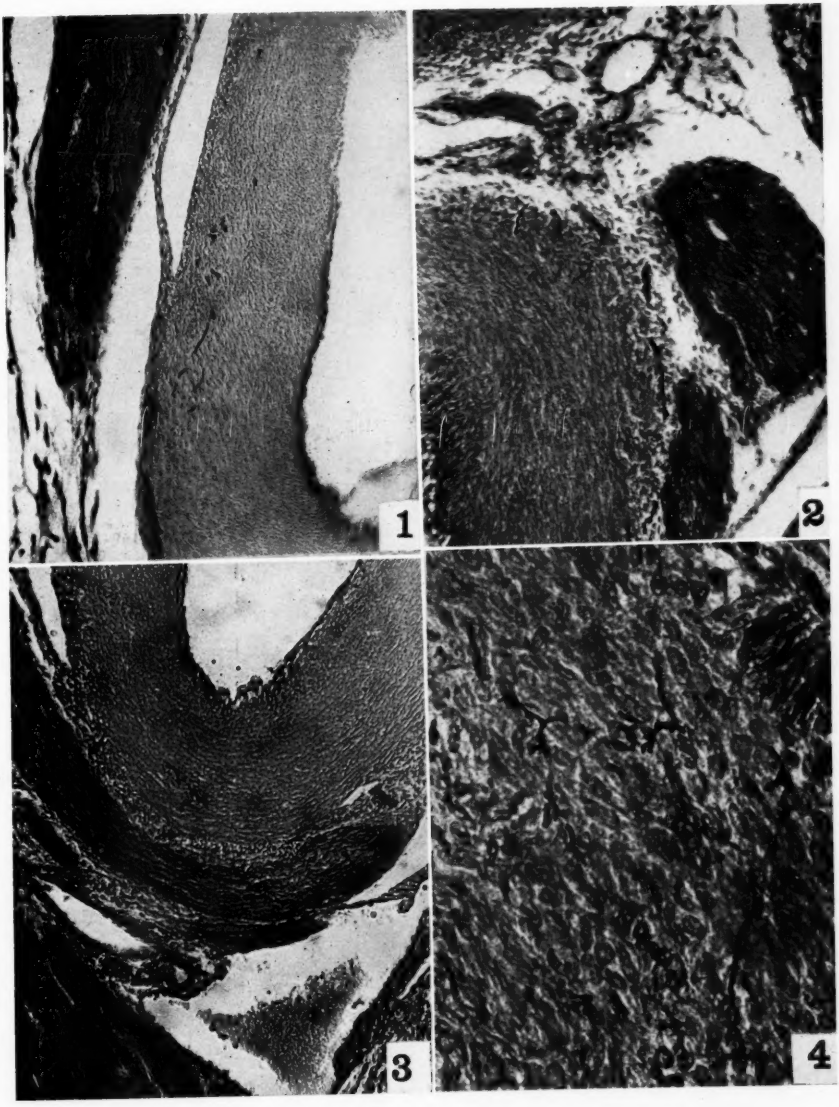
Plate 2

Fig. 5. Nerve fibres and sensory endings in the wall of the ductus arteriosus of a full-term rabbit foetus. A fine fibre can be seen passing among the ramifications of a sensory ending. de Castro technique. ×390.

Fig. 6. Fibres and nerve-endings in the wall of a human ductus arteriosus. Cajal technique. ×350.

Fig. 7. Sensory ending in the ligamentum arteriosum of an adult rabbit. Cajal technique. ×350.

Fig. 8. Portion of a sensory ending in the left pulmonary artery of a full-term rabbit foetus. de Castro technique. ×390.



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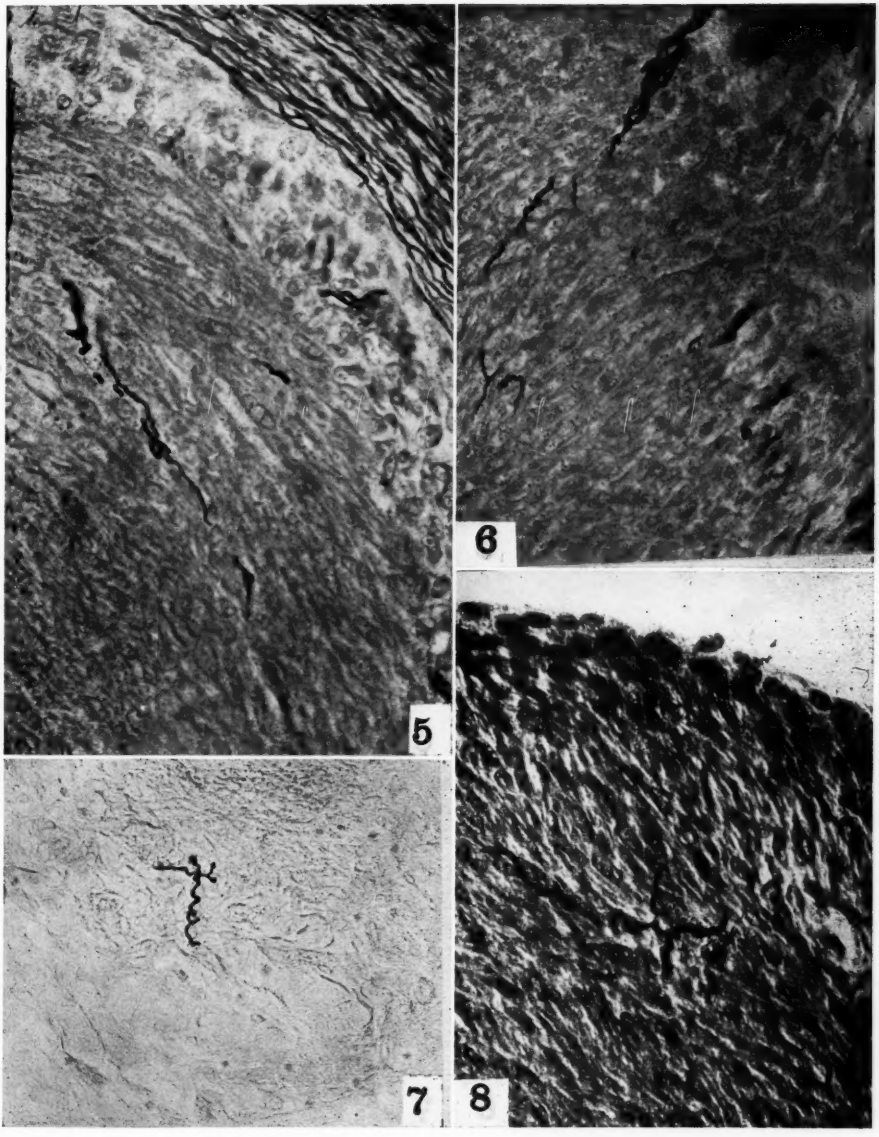

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